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Docket No. 62943/JPW/JML

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

jc886 U.S. PTO
09/648310
08/25/00

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231
BOX: PATENT APPLICATIONS

August 25, 2000

S I R:

Transmitted herewith for filing are the specification and claims of the patent application of:

Paul B. Fisher, Dong-Chul Kang, and Zao-Zhong Su for
Inventor(s)

PROGRESSION SUPPRESSED GENE 13 (PSGen13) AND USES THEREOF
Title of Invention

Also enclosed are:

X 10 sheet(s) of ____informal X formal drawings.

X Oath or declaration of Applicant(s). (unsigned)

X A power of attorney (unsigned)

____ An assignment of the invention to _____

X A Preliminary Amendment

____ A verified statement to establish small entity status under 37 C.F.R. §1.9 and §1.27.

The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE SMALL ENTITY	RATE OTHER ENTITY		FEE SMALL ENTITY	FEE OTHER ENTITY
Total Claims	32 -20	=	12	X	$ 9.00	$18.00	=	$	$ 216
Independent Claims	5 -3	=	2	X	$39.00	$78.00	=	$	$ 156
Multiple Dependent Claims Presented: X Yes ____No					$130.00	$260.00	=	$	$ 260
						BASIC FEE		$	$ 690
						TOTAL FEE		$	$1,322

*If the different in Col. 1 is less than zero, enter "0" in Col. 2

__X__ A check in the amount of $ 1,322.00 to cover the filing fee.

____ Please charge Deposit Account No. _____ in the amount of $_____.

__X__ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with the following or credit any over-payment to Account No. 03-3125:

__X__ Filing fees under 37 C.F.R. §1.16.

__X__ Patent application processing fees under 37 C.F.R. §1.17.

____ The issue fee set in 37 C.F.R. §1.18 at or before mailing of the Notice of Allowance, pursuant to 37 C.F.R. §1.311(b).

__X__ Three copies of this sheet are enclosed.

____ A certified copy of previously filed foreign application No. _____ _____ filed in _____ on _____. Applicant(s) hereby claim priority based upon this aforementioned foreign application under 35 U.S.C. §119.

__X__ Other (identify) One extra loose set of formal drawings and an Express Mail Certificate of Mailing bearing label number EJ 900 852 215 US dated August 25, 2000.

Respectfully submitted,

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Dkt. 0575/62943/JPW/JML

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Paul B. Fisher et al.

U.S. Serial No.: Not Yet Known

Filing date : Herewith

For : PROGRESSION SUPPRESSED GENE 13 (PSGen 13) AND USES THEREOF

1185 Avenue of the Americas
New York, New York 10036
August 25, 2000

Assistant Commissioner for Patents
Washington, D.C. 20231
Box: Patent Application

SIR:

EXPRESS MAIL CERTIFICATE OF MAILING
FOR ABOVE-IDENTIFIED APPLICATION

"Express Mail" mailing label number: EJ 900 852 215 US
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Paul B. Fisher et al.
U.S. Serial No.	:	Not Yet Known
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For	:	PROGRESSION SUPPRESSED GENE 13 (PSGen 13) AND USES THEREOF

1185 Avenue of the Americas
New York, New York 10036
August 25, 2000

Assistant Commissioner for Patents
Washington, D.C. 20231
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SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows:

In the claims:

Please cancel claims 2-8, 11, 12, 23-25, 27-29, 31, 33, 35, 37-39, and 41-43 without prejudice to applicants' right to pursue the subject matter of these claims in a future continuation, divisional or other application.

Thus, claims 1, 9, 10, 13-22, 26, 30, 32, 34, 36 and 40 are pending.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicant's undersigned attorney invites the Examiner to telephone at the number provided below.

No fee is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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Dkt. 0575/62943/JPW/JML

Application
for
United States Letters Patent

To all whom it may concern:

Be it known that **Paul B. Fisher, Dong-Chul Kang, and Zao-Zhong Su**

have invented certain new and useful improvements in

Progression Suppressed Gene 13 (PSGen 13) And Uses Thereof

of which the following is a full, clear and exact description.

Progression Suppressed Gene 13 (PSGen 13) And Uses Thereof

The invention disclosed herein was made with Government support under National Institutes of Health Grant No. CA35675 from the U.S. Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various publications are referenced by author and date within the text. Full citations for these publications may be found listed alphabetically at the end of the specification immediately preceding the claims. All patents, patent applications and publications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Summary of the Invention

The invention provides for an isolated nucleic acid encoding a Progression Suppressed Gene 13 (PSGen 13) protein. The inventional also provides a vector comprising said nucleic acid and a host cell comprising said vector. The invention provides for an isolated Progression Suppressed Gene 13 (PSGen 13) protein. The invention also provides a method for treating cancer in a subject which comprises contacting a cell of the subject with a nucleic acid encoding a Progression Suppressed Gene 13 Protein (PSGen 13) in a sufficient amount so as to cause the cell to express the PSGen 13 protein, thereby treating cancer in the subject. The invention also provides a method for inhibiting growth of a cancer cell which comprises contacting the cancer cell with a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit growth of the cancer cell.

Brief Description of the Figures

Figure 1. Nucleotide and predicted amino acid sequence of the rat PSGen 13 gene (designated PSGen 13). Starting ATG of the open reading frame and stop codon are bold faced and the poly(A) signal is underlined. **(SEQ ID NOS:__ and ___)** ATCC Designation No. _____, which was deposited on August 24, 2000 under provisions of the Budapest Treaty with the American Type Culture Collection (see details hereinbelow).

Figure 2. Nucleotide and predicted amino acid sequence of the human PSGen 13, (designated HuPSGen 13) gene. Starting ATG of the open reading frame and stop codon are bold faced and the poly(A) signal is underlined. ATCC Designation No. _____, which was deposited on August 24, 2000 under provisions of the Budapest Treaty with the American Type Culture Collection (see details hereinbelow). **(SEQ ID NOS: ___ and ___).**

Figure 3. Nucleotide sequence comparison between the rat PSGen 13 and HuPSGen 13 cDNAs. The start and stop codons of the rat PSGen 13 and HuPSGen 13 genes are underlined. **(SEQ ID NOS: ___ and ___).**

Figure 4. Amino acid sequence comparison between the rat PSGen 13 and HuPSGen 13 encoded proteins. Conserved substitutions in the rat PSGen 13 and HuPSGen 13 proteins are underlined. **(SEQ ID NOS: ___ and ___).**

Figure 5. Differential expression of PSGen-13 identified by RSDD and reverse Northern blotting in a large panel of rodent cells displaying differences in transformation progression. A Northern blot of cells displaying various stages of transformation progression was probed with a radiolabeled (^{32}P) rat PSGen 13 cDNA initially identified by RSDD and reverse Northern blotting (6). The cell types used include unprogressed E11 (-), CREF X E11-NMT F1 (-) and CREF X E11-NMT F2 (-) somatic cell hybrids,

E11 X E11-NMT A6 (-) somatic cell hybrid, E11 X E11-NMT 3b (-) somatic cell hybrid, and E11-NMT AZA B1 (-) and E11-NMT AZA C1 (-) 5-azacytidine-treated E11-NMT clones; and progressed E11-NMT (+), CREF X E11-NMT R1 (+) and CREF X E11-NMT R2 (+) somatic cell hybrids, E11 X E11-NMT A6TD (+) nude mouse tumor derived somatic cell hybrid, E11 X E11-NMT IIa (+), E11-Ras R12 (+) and E11-HPV E6/E7 (+) and an E11 clone transformed by the E6 and E7 region of HPV-18. Equal loading of RNAs is demonstrated by ethidium bromide (EtBr) staining. Data from ref. 6.

Figure 6. PSGen 13 suppresses anchorage independent growth in E11-NMT cells. Agar cloning efficiency of the indicated cell types were determined as described previously (7-9). Cell types include, E11, E11-NMT and PSGen 13 transfected E11-NMT clones, designed as NMT-PSG13 cl 3, 5, 6, 7, 8, 9, 10, 11 and 12. Triplicate samples varied by < 10% and replicate assays varied by < 15%.

Figure 7. Northern blotting analysis of rat PSGen 13 and GAPDH expression in E11, E11-NMT and NMT-PSG13 clones. Fifteen micrograms of cellular RNA isolated from the indicated cell types were electrophoresed, transferred to nylon membranes and hybridized with a rat PSGen 13 cDNA and then stripped and probed with GAPDH as previously described (11,12).

Figure 8. Rat PSGen 13 inhibits anchorage independent growth in DU-145 human prostate carcinoma cells. Agar cloning efficiency of the indicated cell types were determined as described previously (7-0). Cell types include, CD-145, DU-145 vector transformed clone (DU-145/Vec) and rat PSGen 13 transfected DU-145 clones, designed as DU-PSG13 cl 11, 12, 13, 14, 15 and 17. Triplicate samples varied by <10% and replicate assays varied by <12%.

Figure 9. Full length PEG-3 promoter-luciferase activity in E11,

E11-NMT and NMT-PSG13 clones. Different cell types were co-transfected with 5 μg of the full length PEG-Prom and 1 μg of a pSV-β-galactosidase plasmid and luciferase activity was determined as described in Materials and Methods 48 hr later. The results are standardized by β-galactosidase activity and represent the average of 3 independent experiments that varied by < 15%.

Figure 10. VEGF promoter-luciferase activity in E11, E11-NMT and NMT-PSG13 clones. Different cell types were co-transfected with 5 μg of the VEGF-Prom-luciferase (12) and 1 μg of a pSV-β-galactosidase plasmid and luciferase activity was determined as described in Materials and Methods 48 hr later. The results are standardized by β-galactosidase activity and represent the average of 3 independent experiments that varied by < 15%.

Detailed Description of the Invention

Throughout this application, references to specific nucleotides are to nucleotides present on the coding strand of the nucleic acid. The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

The invention provides for an isolated nucleic acid encoding a Progression Suppressed Gene 13 (PSGen 13) protein. In one embodiment of the invention, the Progression Suppressed Gene 13 (PSGen 13) protein is a human protein, a rat protein, a primate protein, a mouse protein, or a bovine protein. In another embodiment of the invention, the nucleic acid comprises the polynucleotide sequence shown in SEQ ID NO:1. In another embodiment of the invention, the nucleic acid comprises the polynucleotide sequence shown in SEQ ID NO:2. In another embodiment of the invention, the nucleic acid consists essentially of the polynucleotide sequence shown in SEQ ID NO:1. In another embodiment of the invention, the nucleic acid consists essentially of the polynucleotide sequence shown in SEQ ID NO:2. In another embodiment of the invention, the nucleic acid consists of the polynucleotide sequence shown in SEQ ID NO:1. In another embodiment of the invention, the nucleic acid consists of the polynucleotide sequence shown in SEQ ID NO:2.

The invention provides for a vector comprising the nucleic acid described herein. The invention also provides for a host cell comprising the vector. In one embodiment, the host cell is a tumor cell. In another embodiment, the tumor cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia

cell, a lymphoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

The invention provides for a method for treating cancer in a subject which comprises contacting a cell of the subject with a nucleic acid encoding a Progression Suppressed Gene 13 Protein (PSGen 13) in a sufficient amount so as to cause the cell to express the PSGen 13 protein, thereby treating cancer in the subject. In one embodiment of the invention, the cell is a tumor cell. In another embodiment of the invention, the tumor cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia cell, a lymphoma cell, melanoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

In another embodiment of the invention, the subject is suffering from a form of cancer. In another embodiment of the invention, the form of cancer is melanoma, neuroblastoma, astrocytoma, glioblastoma multiforme, cervical cancer, breast cancer, colon cancer, prostate cancer, osteoscarcoma, chrondosarcoma, a nasopharengeal tumor, a thyroid tumor, a central nervous system tumor, a melanoma , an epithelial tumor, a non-epithelial tumor, a blood tumor, a leukemia, a lymphoma.

In another embodiment of the invention, the contacting is by way of topical application, administration to the subject, injection, electroporation, liposome application, viral-mediated contact, contacting the cell with the nucleic acid, or coculturing the cell with the nucleic acid.

In another embodiment of the invention, the contacting is carried out via injection, oral administration, topical administration,

adenovirus infection, viral-mediated infection, liposome-mediated transfer, topical application to the cells of the subject, or microinjection.

In another embodiment of the invention, the subject is a mammal. In another embodiment of the invention, the mammal is a human.

The invention provides for an isolated Progression Suppressed Gene 13 (PSGen 13) protein. In one embodiment of the invention, the protein is a human protein, a rat protein, a bovine protein, a mouse protein, or a primate protein. In another embodiment of the invention, the protein has a polypeptide sequence which is encoded by the polynucleotide sequence of SEQ ID NO:1. In another embodiment of the invention, the protein has a polypeptide sequence which is encoded by the polynucleotide sequence of SEQ ID NO:2.

The invention provides for an antibody which binds specifically to the protein described herein. In another embodiment of the invention, the antibody is a polyclonal antibody or a monoclonal antibody. In another embodiment of the invention, the antibody is a human antibody, a murine antibody, a primate antibody, a bovine antibody, a sheep antibody or a rat antibody. In another embodiment of the invention, the antibody is a human monoclonal antibody, a humanized murine monoclonal antibody, a humanized primate monoclonal antibody, or a humanized rat monoclonal antibody.

The invention provides for a composition which comprises any one of the nucleic acids described herein. In another embodiment of the invention, the composition is a pharmaceutical composition.

The invention provides for a composition which comprises any one of the proteins described herein and a carrier. In another embodiment of the invention, the composition is a pharmaceutical composition.

The invention provides for a composition which comprises the antibody described herein and a carrier. In another embodiment of the invention, the composition is a pharmaceutical composition.

The invention provides for a method for inhibiting growth of a cancer cell which comprises contacting the cancer cell with a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit growth of the cancer cell. In another embodiment of the invention, the PSGen 13 activator compound comprises a transcription factor which specifically activates expression of a PSGen 13 gene, an agent which prolongs PSGen 13 protein half-life in the cell, or a compound which stabilizes PSGen 13 mRNA in the cell so as to increase translation of PSGen 13 protein in the cell. In another embodiment of the invention, the contacting is by way of topical application, injection, electroporation, liposome application, or coculturing the cell with the nucleic acid. In another embodiment of the invention, the cancer cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia cell, a lymphoma cell, a melanoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

The invention provides for a method for inhibiting angiogenesis associated with tumor growth in a subject which comprises administering to the subject a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit angiogenesis associated with tumor growth in the subject. In another embodiment of the invention, the subject is a mammal. In another embodiment of the invention, the mammal is a human. In another embodiment of the invention, the

subject is suffering from melanoma, neuroblastoma, astrocytoma, glioblastoma multiforme, cervical cancer, breast cancer, colon cancer, prostate cancer, osteoscarcoma, chrondosarcoma, a nasopharengeal tumor, a thyroid tumor, a central nervous system tumor, a melanoma , an epithelial tumor, a non-epithelial tumor, a blood tumor, a leukemia, or a lymphoma.

Biological Deposit

The invention provides for a nucleic acid encoding PSGen 13 (rat) protein, which nucleic acid, designated PSGen 13, ATCC Designation No. _____, deposited August 24, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd, Manassas, Virginia, 20110-2209 under the provision of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. This deposit is an insert within a plasmid vector, pcDNA3.1(+). It is within the EcoRI-Xho I cloning site. The insert is about 0.8 kb in length. The sense strand promoter of the plasmid is T7. The plasmid carries resistance genes to ampicillin and neomycin. The insert origin is EST clone ATCC #2005777. The rat tissue used to isolate the DNA was adrenal gland tissue.

The invention provides for a nucleic acid encoding HuPSGen 13 (human) protein, which nucleic acid, designated HuPSGen 13, ATCC Designation No. _____, deposited August 24, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd, Manassas, Virginia, 20110-2209 under the provision of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. This deposit is an insert within a plasmid vector, pT7T3-Pac. It is within the EcoRI-Not I cloning site. The insert is about 0.83 kb in length. The plasmid carries a resistance gene to ampicillin. The insert origin is EST clone #2525262. The human tissue used to isolate the DNA was human kidney tissue.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology, recombinant DNA technology, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989); DNA Cloning, Vols. I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Animal Cell Culture (R. K. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL press, 1986); Perbal, B., A Practical Guide to Molecular Cloning (1984); the series, Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); and Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell eds., 1986, Blackwell Scientific Publications).

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

In an embodiment of the invention, the host cell is stably transformed with the recombinant expression construct described herein. In another embodiment of the invention, the host cell is a tumor cell. In another embodiment of the invention, the cell is an immortalized cell.

In another embodiment of the invention, the tumor cell is a melanoma cell, a neuroblastoma cell, an astrocytoma cell, a glioblastomoa multifore cell, a cerival cancer cell, a breast cancer cell, a lung cancer cell or a prostate cancer cell.

In another embodiment of the invention, the "introducing" or "contacting" is carried out by a means selected from the group consisting of adenovirus infection, liposome-mediated transfer,

topical application to the cell, and microinjection.

In another embodiment of the invention, the cancer is melanoma, neuroblastoma, astrocytoma, glioblastoma multiforme, cervical cancer, breast cancer, colon cancer, prostate cancer, osteoscarcoma, or chrondosarcoma.

In another embodiment of the invention, the cancer is a cancer of the central nervous system of the subject.

In another embodiment of the invention, the administering is carried out via injection, oral administration, or topical administration.

In another embodiment of the invention, the carrier is an aqueous carrier, a liposome, or a lipid carrier.

Definitions

As used herein "therapeutic gene" means DNA encoding an amino acid sequence corresponding to a functional protein capable of exerting a therapeutic effect on cancer cells or having a regulatory effect on the expression of a function in cells, especially in skin cells (melanocytes).

As used herein "nucleic acid molecule" includes both DNA and RNA and, unless otherwise specified, includes both double-stranded and single-stranded nucleic acids. Also included are hybrids such as DNA-RNA hybrids. Reference to a nucleic acid sequence can also include modified bases as long as the modification does not significantly interfere either with binding of a ligand such as a protein by the nucleic acid or Watson-Crick base pairing.

As used herein "enhancer element" is a nucleotide sequence that increases the rate of transcription of the therapeutic genes or

genes of interest but does not have promoter activity. An enhancer can be moved upstream, downstream, and to the other side of a promoter without significant loss of activity.

Two DNA or polypeptide sequences are "substantially homologous" when at least about 80% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides or amino acids match over a defined length of the molecule. As used herein, "substantially homologous" also refers to sequences showing identity to the specified DNA or polypeptide sequence. DNA sequences that are substantially homologous can be identified in a Southern hybridization, experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al., supra; DNA Cloning, vols I & II, supra; Nucleic Acid Hybridization, supra.

A DNA "coding sequence" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide *in vivo* or *in vitro* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5'-(amino) terminus and a translation stop codon at the 3'-(carboxy) terminus. A coding sequence can include, but is not limited to, procaryotic sequences, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) sources, viral RNA or DNA, and even synthetic nucleotide sequences. A transcription termination sequence will usually be located 3' to the coding sequence.

DNA "control sequences" refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, untranslated regions, including 5'-UTRs and 3'-UTRs, which collectively provide for the transcription and translation of a coding sequence in a host cell.

"Operably linked" refers to an arrangement of nucleotide sequence elements wherein the components so described are configured so as to perform their usual function. Thus, control sequences operably linked to a coding sequence are capable of effecting the expression of the coding sequence. The control sequences need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

A control sequence "directs the transcription" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In procaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. In eucaryotic cells, a stably transformed cell is generally one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication, or one which includes stably maintained extrachromosomal plasmids. This stability is demonstrated by the ability of the eucaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the exogenous DNA.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. An example of a heterologous coding sequence is a construct where

the coding sequence itself (e.g., the coding sequence of PSGen 13 mutant or fragment of nucleic acid encoding PSGen 13) is not found in nature (e.g., synthetic sequences having codons different from the native gene). Likewise, a chimeric sequence, comprising a heterologous structural gene and a gene encoding a PSGen 13 or a portion of such gene, linked to a tissue specific promoter, whether derived from the same or a different gene, will be considered heterologous since such chimeric constructs are not normally found in nature. Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

Vectors

Especially preferred are virus based vectors. In the case of eukaryotic cells, retrovirus or adenovirus based vectors are preferred. Such vectors contain all or a part of a viral genome, such as long term repeats ("LTRs"), promoters (e.g., CMV promoters, SV40 promoter, RSV promoter), enhancers, and so forth. When the host cell is a prokaryote, bacterial viruses, or phages, are preferred. Exemplary of such vectors are vectors based upon, e.g., lambda phage. In any case, the vector may comprise elements of more than one virus.

The resulting vectors are transfected or transformed into a host cell, which may be eukaryotic or prokaryotic. The gene transfer vector of the present invention may additionally comprise a gene encoding a marker or reporter molecule to more easily trace expression of the vector. The particular reporter molecule which can be employed in the present invention is not critical thereto. Examples of such reporter molecules which can be employed in the present invention are well-known in the art and include beta-galactosidase (Fowler et al, Proc. Natl. Acad. Sci., USA, 74:1507 (1977)), luciferase (Tu et al, Biochem., 14:1970 (1975)), and chloramphenicol acetyltransferase (Gorman et al, Mol. Cell Biol., 2:1044-1051 (1982)). The gene transfer vector may contain more

than one gene encoding the same or different foreign polypeptides or RNAs. The gene transfer vector may be any construct which is able to replicate within a host cell and includes plasmids, DNA viruses, retroviruses, as well as isolated nucleotide molecules. Liposome-mediated transfer of the gene transfer vector may also be carried out in the present invention. Examples of such plasmids which can be employed in the present invention include pGL3-based plasmids (Promega). An example of such DNA viruses which can be employed in the present invention are adenoviruses.

Adenoviruses have attracted increasing attention as expression vectors, especially for human gene therapy (Berkner, Curr. Top. Microbiol. Immunol., 158:39-66 (1992)). Examples of such adenovirus serotypes which can be employed in the present invention are well-known in the art and include more than 40 different human adenoviruses, e.g., Ad12 (subgenus A), Ad3 and Ad7 (Subgenus B), Ad2 and Ad5 (Subgenus C), Ad8 (Subgenus D), Ad4 (Subgenus E), Ad40 (Subgenus F) (Wigand et al, In: Adenovirus DNA, Doerfler, Ed., Martinus Nijhoff Publishing, Boston, pp. 408-441 (1986)). Ad5 of subgroup C is the preferred adenovirus employed in the present invention. This is because Ad5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector. Also, adenoviral vectors are commercially available, e.g., pCA3 (Microbix Biosystems Inc.).

Methods for producing adenovirus vectors are well-known in the art (Berkner et al, Nucleic Acids Res., 11:6003-6020 (1983); van Doren et al, Mol. Cell. Biol., 4:1653-1656 (1984); Ghosh-Choudhury et al, Biochem. Biophys. Res. Commun., 147:964-973 (1987); McGrory et al, Virol., 163:614-617 (1988); and Gluzman et al, In: Eurkaryotic Viral Vectors, Ed. Gluzman, Y. pages 187-192, Cold Spring Harbor Laboratory (1982)).

Derivative nucleic acid molecules

Derivative molecules would retain the functional property of the *PSGen 13*, namely, the molecule having such substitutions will still have the same or similar biological activity of PSGen 13 (that is to suppress the cancer phenotype or inhibit growth of cancer cells). Modification is permitted so long as the derivative molecules retain its increased potency compared to a normally occurring PSGen 13 protein. The purpose of the therapeutic gene is to inhibit the growth of cancer cells or produce cytokines or other cytotoxic agents which directly or indirectly inhibit the growth of or kill the cancer cell.

Preferred vectors for use in the methods of the present invention are viral including adenoviruses, retroviral, vectors, adeno-associated viral (AAV) vectors. The viral vector selected should meet the following criteria: 1) the vector must be able to infect the tumor cells and thus viral vectors having an appropriate host range must be selected; 2) the transferred gene should be capable of persisting and being expressed in a cell for an extended period of time; and 3) the vector should be safe to the host and cause minimal cell transformation. Retroviral vectors and adenoviruses offer an efficient, useful, and presently the best-characterized means of introducing and expressing foreign genes efficiently in mammalian cells. These vectors have very broad host and cell type ranges, express genes stably and efficiently. The safety of these vectors has been proved by many research groups. In fact many are in clinical trials.

Other virus vectors that may be used for gene transfer into cells for correction of disorders include retroviruses such as Moloney murine leukemia virus (MoMuLV); papovaviruses such as JC, SV40, polyoma, adenoviruses; Epstein-Barr Virus (EBV); papilloma viruses, e.g. bovine papilloma virus type I (BPV); vaccinia and

poliovirus and other human and animal viruses.

Adenoviruses have several properties that make them attractive as cloning vehicles (Bachettis et al.: Transfer of gene for thymidine kinase-deficient human cells by purified herpes simplex viral DNA. PNAS USA, 1977 74:1590; Berkner, K. L.: Development of adenovirus vectors for expression of heterologous genes. Biotechniques, 1988 6:616; Ghosh-Choudhury G., et al., Human adenovirus cloning vectors based on infectious bacterial plasmids. Gene 1986; 50:161; Hag-Ahmand Y., et al., Development of a helper-independent human adenovirus vector and its use in the transfer of the herpes simplex virus thymidine kinase gene. J Virol 1986; 57:257; Rosenfeld M., et al., Adenovirus-mediated transfer of a recombinant .alpha..sub.1 -antitrypsin gene to the lung epithelium in vivo. Science 1991; 252:431).

For example, adenoviruses possess an intermediate sized genome that replicates in cellular nuclei; many serotypes are clinically innocuous; adenovirus genomes appear to be stable despite insertion of foreign genes; foreign genes appear to be maintained without loss or rearrangement; and adenoviruses can be used as high level transient expression vectors with an expression period up to 4 weeks to several months. Extensive biochemical and genetic studies suggest that it is possible to substitute up to 7-7.5 kb of heterologous sequences for native adenovirus sequences generating viable, conditional, helper-independent vectors (Kaufman R. J.; identification of the component necessary for adenovirus translational control and their utilization in cDNA expression vectors. PNAS USA, 1985 82:689).

AAV is a small human parvovirus with a single stranded DNA genome of approximately 5 kb. This virus can be propagated as an integrated provirus in several human cell types. AAV vectors have several advantage for human gene therapy. For example, they are trophic for human cells but can also infect other mammalian cells; (2) no disease has been associated with AAV in humans or

other animals; (3) integrated AAV genomes appear stable in their host cells; (4) there is no evidence that integration of AAV alters expression of host genes or promoters or promotes their rearrangement; (5) introduced genes can be rescued from the host cell by infection with a helper virus such as adenovirus.

HSV-1 vector system facilitates introduction of virtually any gene into non-mitotic cells (Geller et al. an efficient deletion mutant packaging system for a defective herpes simplex virus vectors: Potential applications to human gene therapy and neuronal physiology. PNAS USA, 1990 87:8950).

Another vector for mammalian gene transfer is the bovine papilloma virus-based vector (Sarver N, et al., Bovine papilloma virus DNA: A novel eukaryotic cloning vector. Mol Cell Biol 1981; 1:486). Vaccinia and other poxvirus-based vectors provide a mammalian gene transfer system. Vaccinia virus is a large double-stranded DNA virus of 120 kilodaltons (kd) genomic size (Panicali D, et al., Construction of poxvirus as cloning vectors: Insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccine virus. Proc Natl Acad Sci USA 1982; 79:4927; Smith et al. infectious vaccinia virus recombinants that express hepatitis B virus surface antigens. Nature, 1983 302:490.)

Retroviruses are packages designed to insert viral genes into host cells (Guild B, et al., Development of retrovirus vectors useful for expressing genes in cultured murine embryonic cells and hematopoietic cells in vivo. J Virol 1988; 62:795; Hock R. A., et al., Retrovirus mediated transfer and expression of drug resistance genes in human hemopoietic progenitor cells. Nature 1986; 320:275). The basic retrovirus consists of two identical strands of RNA packaged in a proviral protein. The core surrounded by a protective coat called the envelope, which is derived from the membrane of the previous host but modified with glycoproteins contributed by the virus.

Markers and amplifiers can also be employed in the subject expression systems. A variety of markers are known which are useful in selecting for transformed cell lines and generally comprise a gene whose expression confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers for mammalian cell lines include, for example, the bacterial xanthine-guanine phosporibosyl transferase gene, which can be selected for in medium containing mycophenolic acid and xanthine (Mulligan et al. (1981) Proc. Natl. Acad. Sci. USA 78:2072-2076), and the aminoglycoside phosphotransferase gene (specifying a protein that inactivates the antibacterial action of neomycin/kanamycin derivatives), which can be selected for using medium containing neomycin derivatives such as G418 which are normally toxic to mammalian cells (Colbere-Garapin et al. (1981) J. Mol. Biol. 150:1-14). Useful markers for other eucaryotic expression systems, are well known to those of skill in the art.

Infection can be carried out in vitro or *in vivo. In vitro* infection of cells is performed by adding the gene transfer vectors to the cell culture medium. When infection is carried out in vivo, the solution containing the gene transfer vectors may be administered by a variety of modes, depending on the tissue which is to be infected. Examples of such modes of administration include injection of gene transfer vectors into the skin, topical application onto the skin, direct application to a surface of epithelium, or instillation into an organ (e.g., time release patch or capsule below the skin or into a tumor).

Expression can be amplified by placing an amplifiable gene, such as the mouse dihydrofolate reductase (dhfr) gene adjacent to the coding sequence. Cells can then be selected for methotrexate resistance in dhfr-deficient cells. See, e.g. Urlaub et al. (1980) Proc. Natl. Acad. Sci. USA 77:4216-4220; Rungold et al. (1981) J. Mol. and Appl. Genet. 1:165-175.

The above-described system can be used to direct the expression of a wide variety of procaryotic, eucaryotic and viral proteins, including, for example, viral glycoproteins suitable for use as vaccine antigens, immunomodulators for regulation of the immune response, hormones, cytokines and growth factors, as well as proteins useful in the production of other biopharmaceuticals.

It may also be desirable to produce mutants or analogs of the PSGen 13 proteins or nucleic acids. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, e.g., Sambrook et al., supra; DNA Cloning, Vols. I and II, supra; Nucleic Acid Hybridization, supra. The mutants would be useful if they, for example, had a prolonged half-life in the tumor cell, thereby having a greater effect on the tumor cell; were more bioavailable; were more easily located to the target cell of interest due to a fused protein or targeting portion; were more efficacious; were more potent; etc. The methods for making mutations and analogs of a known sequenced nucleic acid or protein are well known to one of skill in the art.

In one embodiment of the invention, a heterologous gene sequence, i.e., a therapeutic gene, is inserted into the nucleic acid molecule of the invention. Other embodiments of the isolated nucleic acid molecule of the invention include the addition of a single enhancer element or multiple enhancer elements which amplify the expression of the heterologous therapeutic gene without compromising tissue specificity.

The transformation procedure used depends upon the host to be

transformed. Mammalian cells can conveniently be transformed using, for example, DEAE-dextran based procedures, calcium phosphate precipitation (Graham, F. L. and Van der Eb, A. J. (1973) Virology 52:456-467), protoplast fusion, liposome-mediated transfer, polybrene-mediated transfection and direct microinjection of the DNA into nuclei. Bacterial cells will generally be transformed using calcium chloride, either alone or in combination with other divalent cations and DMSO (Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989)). DNA can also be introduced into bacterial cells by electroporation. Methods of introducing exogenous DNA into yeast hosts typically include either the transformation of spheroplasts or transformation of intact yeast cells treated with alkali cations.

The constructs can also be used in gene therapy or nucleic acid immunization, to direct the production of the desired gene product in vivo, by administering the expression constructs directly to a subject for the in vivo translation thereof. See, e.g. EPA Publication No. 336,523 (Dreano et al., published Oct. 11, 1989). Alternatively, gene transfer can be accomplished by transfecting the subject's cells or tissues with the expression constructs ex vivo and reintroducing the transformed material into the host. The constructs can be directly introduced into the host organism, i.e., by injection (see International Publication No. WO/90/11092; and Wolff et al., (1990) Science 247:1465-1468). Liposome-mediated gene transfer can also be accomplished using known methods. See, e.g., Hazinski et al., (1991) Am. J. Respir. Cell Mol. Biol. 4:206-209; Brigham et al. (1989) Am. J. Med. Sci. 298:278-281; Canonico et al. (1991) Clin. Res. 39:219A; and Nabel et al. (1990) Science 249:1285-1288. Targeting agents, such as antibodies directed against surface antigens expressed on specific cell types, can be covalently conjugated to the liposomal surface so that the nucleic acid can be delivered to specific tissues and cells for local administration.

Human Gene Therapy and Diagnostic Use of Vector

There are several protocols for human gene therapy which have been approved for use by the Recombinant DNA Advisory Committee (RAC) which conform to a general protocol of target cell infection and administration of transfected cells (see for example, Blaese, R.M., et al., 1990; Anderson, W. F., 1992; Culver, K.W. et al., 1991). In addition, U.S. Patent No. 5,399,346 (Anderson, W. F. et al., March 21, 1995, U.S. Serial No. 220,175) describes procedures for retroviral gene transfer. The contents of these support references are incorporated in their entirety into the subject application. Retroviral-mediated gene transfer requires target cells which are undergoing cell division in order to achieve stable integration hence, cells are collected from a subject often by removing blood or bone marrow. It may be necessary to select for a particular subpopulation of the originally harvested cells for use in the infection protocol. Then, a retroviral vector containing the gene(s) of interest would be mixed into the culture medium. The vector binds to the surface of the subject's cells, enters the cells and inserts the gene of interest randomly into a chromosome. The gene of interest is now stably integrated and will remain in place and be passed to all of the daughter cells as the cells grow in number. The cells may be expanded in culture for a total of 9-10 days before reinfusion (Culver et al., 1991). As the length of time the target cells are left in culture increases, the possibility of contamination also increases, therefore a shorter protocol would be more beneficial.

Uses of the Compositions of the Invention

This invention involves targeting a composition which comprises a nucleic acid encoding a PSGen 13 protein; a PSGen 13 protein itself, a fragment, analog or mutant thereof; or a PSGen 13 activator compound (all of which are pharmaceutically acceptable)

to a cancer cell so that the cancer cell's growth is inhibited or reduced or stopped and the cancerous phenotype or condition is ameliorated. The composition which comprises a nucleic acid comprising a sequence encoding PSGen 13 may also include a nucleic acid sequence which is a tissue specific promoter. This promoter, in one embodiment, is specifically active in the cell type which is cell type wherein the subject has cancer. For example, if the subject is suffering from breast cancer, the PSGen 13 nucleic acid sequence could be operably linked to a promoter which will specifically be active (i.e., drive expression of a downstream coding sequence) in breast tissue.

After infecting a susceptible cell, the transgene driven by a specific promoter in the vector expresses the protein encoded by the gene. The use of the highly specific gene vector will allow selective expression of the specific genes in cancer cells.

The present invention provides packaging the cloned genes, i.e. the genes of interest, in such a way that they can be injected directly into the bloodstream or relevant organs of patients who need them. The packaging will protect the foreign DNA from elimination by the immune system and direct it to appropriate tissues or cells.

The methods described below are merely for purposes of illustration and are typical of those that might be used. However, other procedures may also be employed, as is understood in the art. Most of the techniques used to construct vectors and the like are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

General Methods for Vector Construction

Construction of suitable vectors containing the desired

therapeutic gene coding and control sequences employs standard ligation and restriction techniques, which are well understood in the art (see Maniatis et al., in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1982)). Isolated plasmids, DNA sequences, or synthesized oligonucleotides are cleaved, tailored, and religated in the form desired.

Site-specific DNA cleavage is performed by treating with the suitable restriction enzyme (or enzymes) under conditions which are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes (See, e.g. New England Biolabs Product Catalog). In general, about 1 μg of plasmid or DNA sequences is cleaved by one unit of enzyme in about 20 μl of buffer solution. Typically, an excess of restriction enzyme is used to insure complete digestion of the DNA substrate.

Incubation times of about one hour to two hours at about 37 degree. C. are workable, although variations can be tolerated. After each incubation, protein is removed by extraction with phenol/chloroform, and may be followed by ether extraction, and the nucleic acid recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments may be performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in Methods in Enzymology 65:499-560 (1980). Restriction cleaved fragments may be blunt ended by treating with the large fragment of E. coli DNA polymerase I (Klenow) in the presence of the four deoxynucleotide triphosphates (dNTPs) using incubation times of about 15 to 25 min at 20.degree. C. to 25.degree. C. in 50 mM Tris (pH 7.6) 50 mM NaCl, 6 mM MgCl.sub.2, 6 mM DTT and 5-10 .mu.M dNTPs. The Klenow fragment fills in at 5' sticky ends but chews back protruding 3' single strands, even though the four dNTPs are present. If desired, selective repair can be performed by

supplying only one of the dNTPs, or with selected dNTPs, within the limitations dictated by the nature of the sticky ends. After treatment with Klenow, the mixture is extracted with phenol/chloroform and ethanol precipitated. Treatment under appropriate conditions with S1 nuclease or Bal-31 results in hydrolysis of any single-stranded portion.

Ligations are performed in 10-50 μl volumes under the following standard conditions and temperatures using T4 DNA ligase. Ligation protocols are standard (D. Goeddel (ed.) Gene Expression Technology: Methods in Enzymology (1991)). In vector construction employing "vector fragments", the vector fragment is commonly treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase (CIP) in order to remove the 5' phosphate and prevent religation of the vector. Alternatively, religation can be prevented in vectors which have been double digested by additional restriction enzyme digestion of the unwanted fragments.

Suitable vectors include viral vector systems e.g. ADV, RV, and AAV (R. J. Kaufman "Vectors used for expression in mammalian cells" in Gene Expression Technology, edited by D. V. Goeddel (1991).

Many methods for inserting functional DNA transgenes into cells are known in the art. For example, non-vector methods include nonviral physical transfection of DNA into cells; for example, microinjection (DePamphilis et al., BioTechnique 6:662-680 (1988)); liposomal mediated transfection (Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417 (1987), Felgner and Holm, Focus 11:21-25 (1989) and Felgner et al., Proc. West. Pharmacol. Soc. 32: 115-121 (1989)) and other methods known in the art.

Administration of Modified Vectors Into Subject

One way to get DNA into a target cell is to put it inside a

membrane bound sac or vesicle such as a spheroplast or liposome, or by calcium phosphate precipitation (CaPO.sub.4) (Graham F. and Van der Eb, A., Virology 52:456 1973; Schaefer-Ridder M., et al., Liposomes as gene carriers: Efficient transduction of mouse L cells by thymidine kinase gene. Science 1982; 215:166; Stavridis J. C., et al., Construction of transferrin-coated liposomes for in vivo transport of exogenous DNA to bone marrow erythroblasts in rabbits. Exp Cell Res 1986; 164:568-572).

A vesicle can be constructed in such a way that its membrane will fuse with the outer membrane of a target cell. The vector of the invention in vesicles can home into the cancer cells.

The spheroplasts are maintained in high ionic strength buffer until they can be fused through the mammalian target cell using fusogens such as polyethylene glycol.

Liposomes are artificial phospholipid vesicles. Vesicles range in size from 0.2 to 4.0 micrometers and can entrap 10% to 40% of an aqueous buffer containing macromolecules. The liposomes protect the DNA from nucleases and facilitate its introduction into target cells. Transfection can also occur through electroporation. Before administration, the modified vectors are suspended in complete PBS at a selected density for injection. In addition to PBS, any osmotically balanced solution which is physiologically compatible with the subject may be used to suspend and inject the modified vectors into the host.

For injection, the cell suspension is drawn up into the syringe and administered to anesthetized recipients. Multiple injections may be made using this procedure. The viral suspension procedure thus permits administration of genetically modified vectors to any predetermined site in the skin, is relatively non-traumatic, allows multiple administrations simultaneously in several different sites or the same site using the same viral suspension. Multiple injections may consist of a mixture of therapeutic

genes.

Survival of the Modified Vectors So Administered

Expression of a gene is controlled at the transcription, translation or post-translation levels. Transcription initiation is an early and critical event in gene expression. This depends on the promoter and enhancer sequences and is influenced by specific cellular factors that interact with these sequences. The transcriptional unit of many prokaryotic genes consists of the promoter and in some cases enhancer or regulator elements (Banerji et al., Cell 27:299 (1981); Corden et al., Science 209:1406 (1980); and Breathnach and Chambon, Ann. Rev. Biochem. 50:349 (1981)). For retroviruses, control elements involved in the replication of the retroviral genome reside in the long terminal repeat (LTR) (Weiss et al., eds., In: The molecular biology of tumor viruses: RNA tumor viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982)).

Moloney murine leukemia virus (MLV) and Rous sarcoma virus (RSV) LTRs contain promoter and enhancer sequences (Jolly et al., Nucleic Acids Res. 11:1855 (1983); Capecchi et al., In: Enhancer and eukaryotic gene expression, Gulzman and Shenk, eds., pp. 101-102, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.).

Promoter and enhancer regions of a number of non-viral promoters have also been described (Schmidt et al., Nature 314:285 (1985); Rossi and de Crombrugghe, Proc. Natl. Acad. Sci. USA 84:5590-5594 (1987)).

The present invention provides methods for maintaining and increasing expression of therapeutic genes using a skin specific promoter.

In addition to using viral and non-viral promoters to drive

therapeutic gene expression, an enhancer sequence may be used to increase the level of therapeutic gene expression. Enhancers can increase the transcriptional activity not only of their native gene but also of some foreign genes (Armelor, Proc. Natl. Acad. Sci. USA 70:2702 (1973)).

Therapeutic gene expression may also be increased for long term stable expression after injection using cytokines to modulate promoter activity.

The methods of the invention are exemplified by preferred embodiments in which modified vectors carrying a therapeutic gene are injected intracerebrally into a subject.

The most effective mode of administration and dosage regimen for the molecules of the present invention depends upon the exact location of the cancer being treated, the severity and course of the cancer, the subject's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the molecules should be titrated to the individual subject. The molecules may be delivered directly or indirectly via another cell, autologous cells are preferred, but heterologous cells are encompassed within the scope of the invention.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m.sup.2 of surface area is described by Freireich, E. J., et al. Cancer Chemother., Rep. 50 (4):219-244 (1966). Adjustments in the dosage regimen may be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses may be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided dose may be administered daily or proportionally reduced depending on the specific therapeutic situation).

It would be clear that the dose of the molecules of the invention

required to achieve cures may be further reduced with schedule optimization.

This invention is illustrated in the Experimental Details section which follows. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

EXPERIMENTAL DETAILS

Example 1: Progression Suppressed Gene 13 (PSGen 13) Inhibits the Transformed State in Rodent and Human Cancer Cells

A reciprocal subtraction differentiation RNA display (RSDD) approach has been developed that permits the efficient identification and cloning of differentially expressed genes. RSDD has been applied to cancer progression to identify genes with enhanced expression in unprogressed and progressed tumor cells. Using adenovirus transformed rodent cells displaying stable changes in expression of the transformed phenotype; RSDD permitted the cloning of an expressed sequence tag (EST) designated PSGen 13 that displays elevated expression in unprogressed versus progressed transformed cells. A full-length rodent PSGen 13 gene was generated, placed in an expression vector and stably transfected into a progressed rodent transformed cell line, E11-NMT, and the DU-145 human prostate carcinoma cell line. A series of random single cell clones were isolated and evaluated for expression of the transformed state as documented by their ability to grow in an anchorage independent manner. Specific E11-NMT and DU-145 clones transfected with the rat PSGen 13 expression vector, but not the control expression vector lacking the rat PSGen 13 gene, were inhibited in their ability to grow in an anchorage independent manner. In the case of E11-NMT transformants transfected with rat PSGen 13, transcription of a specific progression elevated gene, PEG-3, and the vascular endothelial growth factor (VEGF) gene was inhibited. Using the rat PSGen 13 sequence, a human PSGen 13, HuPSGen 13, gene with significant DNA and protein sequence homology, 75 and 94%, respectively, to the rat PSGen 13 gene has been identified. The present studies demonstrate that PSGen 13 is a suppressor of the cancer phenotype and suppression may involve changes in the expression of cancer progression inducing and angiogenesis stimulating genes. In these contexts, PSGen 13 may prove amenable for the gene-based therapy of human cancer.

OVERVIEW

Cancer is a progressive disease involving changes in the expression of genes that either stimulate or inhibit the carcinogenic process (1-5). Using RSDD in combination with a rodent cancer progression model, several known and novel genes displaying either elevated, progression-elevated gene (PSGen), or reduced expression, progression-suppressed gene (PSGen), were identified and cloned (6). Studies have currently focused on PSGen 13 which is expressed at higher levels in rodent cancer cells displaying a non-progressed transformed phenotype (6), i.e., low growth efficiency in agar and extended tumor latency times in animals (7-10). PSGen 13 was initially identified as a novel gene sequence without homology to previously expressed genes (6). A full-length rat PSGen 13, rat PSGen 13, gene was produced and evaluated for biological activity in rodent and human tumor cells. These studies demonstrate that stable expression of rat PSGen 13 in a progressed adenovirus type 5-transformed Sprague-Dawley rat embryo clone, E11-NMT, results in a suppression of the progression phenotype. Suppression is associated with a reduction in anchorage independent growth to a level that is apparent in unprogressed, E11, transformants. Moreover, progression suppressed transformed clones also display a reduction in transcription of the progression elevated gene, PEG-3 (11,12), and VEGF (12). In DU-145 human prostate cancer cells, rat PSGen 13 transfected clones have also been identified that display a reduction in anchorage independence. These findings suggest that rat PSGen 13 can functionally alter the state of progression of rodent and human tumor cells. In addition, on the basis of the rat PSGen 13 sequence a human PSGen 13, HuPSGen 13, gene with significant homology to the rodent gene has been isolated.

MATERIALS AND METHODS

Cell cultures and agar growth assays. E11 is a single cell clone

of H5ts125-transformed Sprague-Dawley secondary RE cells (13). E11-NMT is a subclone of E11 cells derived from a nude mouse tumor induced by the E11 cell line (10). R12 is a Ha-ras oncogene transformed E11 clone (14). F1 and F2 are suppressed somatic cell hybrids with a flat morphology that were formed between E11-NMT and CREF cells (15). R1 and R2 are progressed somatic cell hybrids with a round morphology that were created by fusing E11-NMT and CREF cells (15). E11-HPV E6/E7 is a clone of E11 cells transformed with E6/E7 gene region of human papilloma virus type 18 (11). E11 x E11-NMT A6 and 3b are independent somatic cell hybrid clones formed between E11 and E11-NMT cells that do not display the progression phenotype (16). E11 X E11-NMT A6TD is a progressed somatic cell hybrid formed by isolating a tumor induced in a nude mouse by the E11 X E11-NMT A6 somatic cell hybrid (11,16). E11 X E11-NMT IIa is a somatic cell hybrid formed between E11 and E11-NMT that exhibits the progression phenotype (16). E11-NMT Aza B1 and Aza C1 are independent clones of E11-NMT cells treated with 5-azacytidine and displaying suppression in the progression phenotype (11,16). CREF is a specific immortal non-transformed and non-tumorigenic clone of Fischer rat embryo fibroblast cells (17). DU-145 is a hormone refractive human prostate carcinoma cell line (18). All cultures were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% FBS (DMEM-5) at 37°C in a humidified 5% CO_2 95% air incubator. Anchorage independent growth assays were performed as described previously by seeding variable number of cells in 0.4% noble agar containing medium on a base layer of 0.8% noble agar containing medium (6-13).

Construction of PSGen 13 expressing E11-NMT clones. E11-NMT and DU-145 cells were transfected with a pcDNA3.1 (+) expression vector (containing a neomycin resistance gene) lacking or having the complete PSGen 13 gene as previously described (11). Briefly, 1 X 105 cells were seeded in 10-cm tissue culture plates, 6 hr later 10 _g of purified pcDNA3.1 (+) vector or a rat PSGen 13/pcDNA3.1 (+) construct was incubated with 30 μl of

Lipofectamine (Gibco BRL) and this mixture was added to cells for 8 hr. The next day the media was changed with the addition of 500 μg/ml of G418 and media was changed 2X per week for three weeks. G418-resistant colonies were isolated using cloning cylinders and maintained as independent cell lines, referred to as NMT-PSG13 clones (cl 3, 5, 6, 7, 8, 9, 10, 11 and 12) and DU-PSG13 clones (cl 11, 12, 13, 14, 15 and 17), in complete media containing 100 μg/ml of G418. Additionally, NMT-vector and DU-145-Vec clones were isolated and maintained as independent cell lines in complete media containing 100 μg/ml of G418.

Northern blotting assays. Total cellular RNA was isolated by the guanidinium/phenol extraction method and Northern blotting was performed as described (11). Fifteen μg of RNA were denatured and electrophoresed in 1.2% agarose gels with 3% formaldehyde, transferred to nylon membranes and hybridized sequentially with a ^{32}P-labeled rat PSGen 13 cDNA probe, the blot was stripped and then reprobed with a ^{32}P-labeled GAPDH cDNA probe as described previously (11). Following hybridization, the filters were washed and exposed for autoradiography.

Promoter analyses. To evaluate the activity of the full length rat PEG-3 promoter (PEG-Prom) and VEGF-Promoter constructs, cells (E11, E11-NMT and NMT-PSGen13) clones, were seeded at 2 X 10^5 / 35-mm tissue culture plate and ~24 h later transfected with 5 μg of the PEG-Prom-luciferase or VEGF-Prom-Luciferase constructs plus 1 μg of SV40-β-gal Vector (Promega) mixed with 10 μl of Lipofectamine Reagent (Gibco) in 200 μl of serum-free media. After 20 min at RT, 800 μl of serum-free media were added resulting in a final volume of 1 ml. The transfection mixture was removed after 14 hr and the cells were washed 3X with serum-free media and incubated at 37oC for an additional 48 hr in complete growth media. Cells were harvested and lysed to make extracts (19) utilized in β-gal and Luciferase reporter assays. Luminometric determinations of Luciferase and β-gal activity was

performed using commercial kits (Promega and Tropix, respectively). For Luciferase assays, 10 μl of cell lysate were mixed with 40 μl of Luciferase Assay substrate (Promega). For β-gal assays, 10 μl of the cell lysate were mixed with 100 μl of diluted Galecton-Plus with 150 μl of Accelerator (Tropix). Promoter analysis data were collected a minimum of three times using triplicate samples for each experimental point and the data was standardized with the β-gal data.

Cloning a full length rat PSGen 13 and a HuPSGen 13 cDNA. An original rat PSGen 13 EST was identified using RSDD and reverse Northern hybridization as a gene displaying elevated expression in E11 versus E11-NMT cells (6). A full length open reading frame (ORF) of rat PSGen 13 was cloned using the complete open reading frame (C-ORF) approach with gene specific primers (20) and electronic data mining based on the EST sequence. Primers used for C-ORF were PSGen13-R2 (TCG CTT CTC ACT TTG ACG GAG TGT CAA G) **(SEQ ID NO: __)** and PSGen13-R2 Nested (TGT CAA GTG TGG CAG AGA CTA AGA ATG G) **(SEQ ID NO: __)**. In addition, full length rat PSGen 13 and HuPSGen 13 cDNA clones were identified by sequence comparison of the rat PSGen 13 EST sequence with GenBank by BLAST. Selected clones (ATCC #2005777 from rat PSGen 13 and ATCC #2525262 for HuPSGen13) were procured (Research Genetics) and sequenced.

Experimental Results

Sequence Informatics of PSGen 13. The cloned full length rat PSGen 13 cDNA consists of 780 bp excluding the poly(A) tail. A poly(A) signal (AATAAA) is located at position 763 (Fig. 1) **(SEQ ID NO:___)**. The ORF starts at the first ATG at 170 bp followed by in-frame stop codon at 86 bp and spans to 415 bp. Rat PSGen 13 encodes a protein with predicted 81 amino acids of calculated molecular weight of 9 kDa with a pI of 5.52 (Fig. 1). Protein sequence analysis did not indicate hydrophobic patches for

membrane spanning regions or signal peptide sequences characteristic of secretory proteins. Motif and pattern analysis also failed to identify sequence homologies with previously reported genes, information that is useful in providing potential insights into the biological function and or mode of action of rat PSGen 13. Based on this observation, rat PSGen 13 appears to encode a novel class of proteins.

A human homologue of Rat PSGen 13 (HuPSGen 13) was electronically cloned by analyzing sequences reported in the GenBank data base (Fig. 2) **(SEQ ID NO:___)**. HuPSGen 13 is 75% identical to rat PSGen 13 at the nucleotide level, but 94% identical to Rat PSGen 13 on a protein level (79/81) (Figs. 3 and 4). Of the 5 residues that are distinct in HuPSGen 13, three of them (D at 4, K at 38 and I at 77) are conserved substitutions of rat PSGen 13 (E at 4, R at 38 and V at 77, respectively), which suggests strong conservation in functionality. Furthermore, sequence identity of HuPSGen 13 with rat PSGen 13 protein is 87% at the nucleotide level. Both 5' and 3' untranslated regions display 68.7% and 68.3% identity, respectively, and are more diverse between rat PSGen 13 and HuPSGen 13 than the ORF, which is not uncommon between interspecies homologues. Considering the degree of conservation in the ORF and resulting protein sequence, HuPSGen is an orthologue of rat PSGen 13. The cloned HuPSGen 13 cDNA consists of 835 bp excluding the poly(A) tail and a canonical poly(A) signal was observed at 814 bp. Although an in-frame stop codon was not present, the ORF of HuPSGen 13 starts at the first ATG (197 bp) and runs through 442 bp. HuPSGen 13 encodes 81 amino acids of calculated Mol. Wt. of 9 kDa with a pI of 5.86. As in rat PSGen 13, computational protein sequence analysis did not yield any known functional motifs.

Rat PSGen 13 Suppresses Anchorage Independent Growth in E11-NMT and DU-145 Cells. Rat PSGen 13 was identified using RSDD as a gene displaying elevated expression in E11 cells versus E11-NMT cells (6). Using a panel of rodent cell lines displaying either

a progressed phenotype (+), as indicated by elevated growth in agar and short tumor latency times, or unprogressed (-), as indicated by reduced growth in agar and extended tumor latency times, a direct correlation between reduced rat PSGen 13 expression and the progression phenotype was found (Fig. 5) (6). The level of rat PSGen 13 was elevated in E11, CREF X E11-NMT F1 and F2, E11 X E11-NMT A6, and E11-NMT AZA B1 cells which do not display the progression phenotype. In contrast, rat PSGen 13 expression was lower in progressed E11-NMT, CREF X E11-NMT R1 and R2, E11 X E11-NMT A6TD, E11 X E11-NMT IIa, E11-Ras R12 and E11-HPV E6/E7 cells. These findings documented a potential inverse relationship between expression of the progression phenotype and rat PSGen 13 expression. They also raised the possibility that PSGen 13 might play a functional role in the progression process.

A full-length rat PSGen 13 was isolated using the C-ORF procedure (20), cloned into an expression vector and stably transfected into E11-NMT and DU-145 cells. Random clones were isolated and evaluated for their ability to form macroscopic colonies when seeded in semi-solid agar (7-9). E11 cells grow with a low efficiency in agar, whereas progressed E11-NMT cells display elevated growth in agar, forming more and larger colonies than E11 cells (Fig. 6) (7-10). Analysis of the random NMT-PSGen 13 clones, designated as NMT-PSG13 cl number, indicated specific clones displaying no significant reduction in anchorage independence, i.e., NMT-PSG13 cl 3 and 5, and clones displaying reduced agar cloning efficiencies similar to E11 cells, i.e., NMT-PSG13 cl 7 and 12. In addition, a number of clones were also identified that display a significant reduction in anchorage independence versus E11-NMT cells, but less growth suppression than NMT-PSG13 cl 7 and 12, including NMT-PSG13 cl 6, 8, 9, 10, and 11. To confirm that suppression of anchorage independence was associated with increased expression of PSGen 13, total RNA was isolated from E11, E11-NMT and NMT-PSG13 cl 3, 5, 7 and 12 and evaluated by Northern blotting (Fig. 7). This experiment

documented expression of PSGen 13 in E11 and NMT-PSG13 cl 7 and 12, but not in E11-NMT or NMT-PSG13 cl 3 and 5. These results demonstrate that forced expression of PSGen-13 in E11-NMT cells results in a suppression of anchorage independent growth, a marker of the progression phenotype in this transformation model system.

To determine if the rat PSGen 13 gene could effect the transformed phenotype in human cancer cells, a full-length Rat PSGen 13 cDNA was transfected into DU-145 human prostate cancer cells and random clones were isolated. The clones were then evaluated for anchorage independent growth (Fig. 8). Several clones were identified, including DU-PEG13 cl 11, 12 and 14, that displayed a significant reduction in anchorage independent growth in comparison with DU-145 cells. Additional clones, including a vector transfected clone and DU-PEG13 cl 13, 15 and 17 displayed similar cloning efficiencies in agar as untransfected DU-145 cells. These results demonstrate that rat PSGen 13 can also suppress the transformed phenotype in human cancer cells, thereby indicating a more general inhibitory capacity of this gene product in tumor cells.

Rat PSGen 13 Inhibits Transcriptional Activity in E11-NMT Cells. Previous studies indicate that E11-NMT cells display elevated transcription of the PEG-3 and VEGF genes as compared to E11 cells (12). These changes in gene expression are believed to be important determinants of the aggressive progressed cancer phenotype of E11-NMT versus E11 cells (12). To determine if rat PSGen 13 expression can effect these important progression related genes, the transcriptional activities of PEG-Prom-Luciferase and VEGF-Prom-Luciferase constructs were evaluated in E11, E11-NMT and the different NMT-PSG13 clones (Figs. 9 and 10). As previously reported (12), the PEG-Prom and VEGF-Prom were more active in E11-NMT than in E11 cells. Both NMT-PSG13 cl 7 and 12 displayed a reduction in PEG-Prom activity similar to that of E11 cells (Fig. 9). In contrast, when

compared with E11-NMT cells the levels of PEG-Prom-Luciferase activity were unchanged in NMT-PSG13 cl 3 and 5, which do not express elevated Rat PSGen 13 mRNA (Fig. 7). Although of lesser magnitude than observed in NMT-PSG13 cl 7 and 12, reductions in the level of PEG-Prom activity were also apparent in NMT-PSG13 cl 6 (smallest reduction), 8, 9, 10 and 11. In the case of the VEGF-Prom, no change in promoter activity was apparent in NMT-PSG13 cl 3 and 5 versus E11-NMT cells (Fig. 10). However, a similar reduction in VEGF-Prom activity, approaching that observed in E11 cells, was apparent in NMT-PSG13 cl 7, 8, 9, 10, 11 and 12. These results suggest that expression of PSGen 13 can regulate expression of both PEG-3 and VEGF. It is not presently known if this regulation of the transcriptional activity of either of these two genes occurs by a direct or indirect mechanism.

Conclusions

The RSDD approach has successfully identified a novel gene, Rat PSGen 13, that can functionally regulate cancer progression in both rodent and human tumor cells. Evidence is presented that forced expression of Rat PSGen 13 in rodent tumor cells displaying an aggressive cancer phenotype, E11-NMT, results in a suppression of the cancer phenotype as monitored by a reduction in anchorage independent growth. Similarly, when overexpressed in a human prostate cancer cell line (DU-145), rat PSGen 13 also induces an inhibition in anchorage independence. The mechanism by which PSGen 13 induces its cancer growth suppression properties is not known. However, in the E11-NMT model forced expression of rat PSGen 13 directly correlates with a suppression in the transcriptional activities of two significant cancer progression regulating genes, PEG-3 and VEGF. Based on sequence homology, a human PSGen 13 gene, HuPSGen 13, has been isolated. Since this gene is highly homologous to the rat PSGen 13 gene, 75% and 94% on a nucleotide and protein level, respectively, there is a high probability that the HuPSGen 13 gene will behave

biologically in a similar manner as the rat gene, i.e., it will function as a suppressor of the cancer phenotype and tumor progression. Although further studies are required, the present studies provide initial support for the potential use of PSGen 13 for the targeted therapy of human cancer.

References

1. Fisher, P.B. (1984). In:Tumor Promotion and Cocarcinogenesis In Vitro, Mechanisms of Tumor Promotion. Slaga T. J. (ed). CRC Press, Inc., Boca Raton, FL, pp. 57-123.
2. Bishop, J.M. (1991). Cell, 64,235-248.
3. Knudson, A.G. (1993). Proc. Natl. Acad. Sci. USA, 90, 10914-10921.
4. Levine, A.J. (1993). Annu. Rev. Biochem., 62, 623-651.
5. Vogelstein, B. & Kinzler, K.W. (1993). Trends Genet., 9, 138-141.
6. Kang, D.-c., LaFrance, R., Su, Z.-z. & Fisher, P. B. (1998) Proc. Natl. Acad. Sci. USA, 95, 13788-13793.
7. Fisher, P.B., Goldstein, N.I. & Weinstein, I.B. (1979a). Cancer Res., 39, 3051-3057.
8. Fisher, P.B., Dorsch-Hasler, K., Weinstein, I.B. & Ginsberg, H.S. (1979b). Nature, 281, 591-594.
9. Fisher, P.B., Bozzone, J.H. & Weinstein, I.B. (1979c). Cell, 18, 695-705.
10. Babiss, L. E., Zimmer, S.G. & Fisher, P.B. (1985). Science, 228,1099-1101.
11. Su, Z.-z., Shi, Y. & Fisher, P.B. (1997) Proc. Natl. Acad. Sci. USA, 94, 9125-9130.
12. Su, Z.-z., Goldstein, N.I., Jiang, H., Wang, M.-N., Duigou, G.J., Young, C.S.H. & Fisher, P.B. (1999) Proc. Natl. Acad. Sci. USA, 96, 15115-15120.
13. Fisher, P.B., Weinstein, I.B., Eisenberg, D. & Ginsberg, H.S. (1978). Proc. Natl. Acad. Sci. USA, 75, 2311-2314.
14. Duigou, G.J., Babiss, L.E. & Fisher, P.B. (1989). NY Acad. Sci., 567, 302-306.
15. Duigou, G.J., Babiss, L.E., Iman, D.S., Shay, J.W. & Fisher, P.B. (1990). Mol. Cell. Biol., 10, 2027-2034.
16. Reddy, P.G., Su, Z.-z. & Fisher, P.B. (1993). In: Chromosome and Genetic Analysis, Methods in Molecular Genetics. Adolph K. W. (ed). Vol 1. Academic Press, Inc., Orlando, FL, pp. 68-102.

17. Fisher, P.B., Babiss, L.E., Weinstein, I.B. & Ginsburg, H.S. (1982). Proc. Natl. Acad. Sci. USA, 79, 3527-3531.

18. Jiang, H., Su, Z.-z., Lin, J.J., Goldstein, N. I., Young, C. S. H. & Fisher, P. B. (1996) Proc. Natl. Acad. Sci. USA, 93, 9160-9165.

19. Gopalkrishnan, R.V., Christiansen, K., Goldstein, N.I., DePinho, R.A. & Fisher, P.B. (1999). Nucl. Acids Res., .27, 4775-4782.

20. Kang, D.-c. & Fisher, P.B. (2000). Unpublished data.

What is claimed is:

1. An isolated nucleic acid encoding a Progression Suppressed Gene 13 (PSGen 13) protein.

2. The isolated nucleic acid of claim 1, wherein the Progression Suppressed Gene 13 (PSGen 13) protein is a human protein, a rat protein, a primate protein, a mouse protein, or a bovine protein.

3. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the polynucleotide sequence shown in SEQ ID NO:1.

4. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises the polynucleotide sequence shown in SEQ ID NO:2.

5. The isolated nucleic acid of claim 1, wherein the nucleic acid consists essentially of the polynucleotide sequence shown in SEQ ID NO:1.

6. The isolated nucleic acid of claim 1, wherein the nucleic acid consists essentially of the polynucleotide sequence shown in SEQ ID NO:2.

7. The isolated nucleic acid of claim 1, wherein the nucleic acid consists of the polynucleotide sequence shown in SEQ ID NO:1.

8. The isolated nucleic acid of claim 1, wherein the nucleic acid consists of the polynucleotide sequence shown in SEQ ID NO:2.

9. A vector comprising the nucleic acid of any one of claims

1 to 8.

10. A host cell comprising the vector of claim 9.

11. The host cell of claim 10, wherein the host cell is a tumor cell.

12. The host cell of claim 11, wherein the tumor cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia cell, a lymphoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

13. A method for treating cancer in a subject which comprises contacting a cell of the subject with a nucleic acid encoding a Progression Suppressed Gene 13 Protein (PSGen 13) in a sufficient amount so as to cause the cell to express the PSGen 13 protein, thereby treating cancer in the subject.

14. The method of claim 13, wherein the cell is a tumor cell.

15. The method of claim 14, wherein the tumor cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia cell, a lymphoma cell, melanoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

16. The method of claim 13, wherein the subject is suffering from a form of cancer.

17. The method of claim 16, wherein the form of cancer is melanoma, neuroblastoma, astrocytoma, glioblastoma multiforme, cervical cancer, breast cancer, colon cancer, prostate cancer, osteoscarcoma, chrondosarcoma, a nasopharengeal tumor, a thyroid tumor, a central nervous system tumor, a melanoma , an epithelial tumor, a non-epithelial tumor, a blood tumor, a leukemia, a lymphoma.

18. The method of claim 13, wherein the contacting is by way of topical application, administration to the subject, injection, electroporation, liposome application, viral-mediated contact, contacting the cell with the nucleic acid, or coculturing the cell with the nucleic acid.

19. The method of claim 18, wherein the contacting is carried out via injection, oral administration, topical administration, adenovirus infection, viral-mediated infection, liposome-mediated transfer, topical application to the cells of the subject, or microinjection.

20. The method of claim 13, wherein the subject is a mammal.

21. The method of claim 20, wherein the mammal is a human.

22. An isolated Progression Suppressed Gene 13 (PSGen 13) protein.

23. The protein of claim 22, wherein the protein is a human protein, a rat protein, a bovine protein, a mouse protein, or a primate protein.

24. The protein of claim 22, wherein the protein has a polypeptide sequence which is encoded by the polynucleotide sequence of SEQ ID NO:1.

25. The protein of claim 22, wherein the protein has a polypeptide sequence which is encoded by the polynucleotide sequence of SEQ ID NO:2.

26. An antibody which binds specifically to the protein of any one of claims 22 to 25.

27. The antibody of claim 26, wherein the antibody is a polyclonal antibody or a monoclonal antibody.

28. The antibody of claim 26, wherein the antibody is a human antibody, a murine antibody, a primate antibody, a bovine antibody, a sheep antibody or a rat antibody.

29. The antibody of claim 26, wherein the antibody is a human monoclonal antibody, a humanized murine monoclonal antibody, a humanized primate monoclonal antibody, or a humanized rat monoclonal antibody.

30. A composition which comprises any one of the nucleic acids of claims 1 to 8 and a carrier.

31. The composition of claim 30, wherein the composition is a pharmaceutical composition.

32. A composition which comprises any one of the proteins of claims 22 to 25 and a carrier.

33. The composition of claim 32, wherein the composition is a pharmaceutical composition.

34. A composition which comprises the antibody of claim 26 and a carrier.

35. The composition of claim 34, wherein the composition is a pharmaceutical composition.

36. A method for inhibiting growth of a cancer cell which comprises contacting the cancer cell with a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit growth of the cancer cell.

37. The method of claim 36, wherein the PSGen 13 activator compound comprises a transcription factor which specifically activates expression of a PSGen 13 gene, an agent which prolongs PSGen 13 protein half-life in the cell, or a compound which stabilizes PSGen 13 mRNA in the cell so as to increase translation of PSGen 13 protein in the cell.

38. The method of claim 36, wherein the contacting is by way of topical application, injection, electroporation, liposome application, or coculturing the cell with the nucleic acid.

39. The method of claim 36, wherein the cancer cell is a nasopharengeal tumor cell, a thyroid tumor cell, a central nervous system tumor cell, a melanoma cell, an epithelial tumor cell, a non-epithelial tumor cell, a blood tumor cell, a leukemia cell, a lymphoma cell, a melanoma cell, a neuroblastoma cell, a cervical cancer cell, a breast cancer cell, a lung cancer cell, a prostate cancer cell, a colon cancer cell or a glioblastoma multiforme cell.

40. A method for inhibiting angiogenesis associated with tumor growth in a subject which comprises administering to the subject a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit angiogenesis associated with tumor growth in the subject.

41. The method of claim 40, wherein the subject is a mammal.

42. The method of claim 41, wherein the mammal is a human.

43. The method of claim 40, wherein the subject is suffering from melanoma, neuroblastoma, astrocytoma, glioblastoma multiforme, cervical cancer, breast cancer, colon cancer, prostate cancer, osteoscarcoma, chrondosarcoma, a nasopharengeal tumor, a thyroid tumor, a central nervous system tumor, a melanoma , an epithelial tumor, a non-epithelial tumor, a blood tumor, a leukemia, or a lymphoma.

Dkt. 0575/62943/JPW/JML

Progression Suppressed Gene 13 (PSGen 13) And Uses Thereof

Abstract of the Disclosure

The invention provides for an isolated nucleic acid encoding a Progression Suppressed Gene 13 (PSGen 13) protein. The inventional also provides a vector comprising said nucleic acid and a host cell comprising said vector. The invention provides for an isolated Progression Suppressed Gene 13 (PSGen 13) protein. The invention also provides a method for treating cancer in a subject which comprises contacting a cell of the subject with a nucleic acid encoding a Progression Suppressed Gene 13 Protein (PSGen 13) in a sufficient amount so as to cause the cell to express the PSGen 13 protein, thereby treating cancer in the subject. The invention also provides a method for inhibiting growth of a cancer cell which comprises contacting the cancer cell with a pharmaceutical composition comprising a nucleic acid encoding a PSGen 13 protein, a PSGen 13 protein or a PSGen 13 activator compound in a sufficient amount so as to inhibit growth of the cancer cell.

Figure 1

```
GGCACGAGCTCTCCTCGTCCCCTCCCTTCTCCACTGCAGCCTTTCTCTTAGCCCGAACCA 60
CTTCCTTCTTCTGCTTGTTCCTCCCTAGGGCGCGGAAGCTGAGTGCAGGGTTCAGACCCA 120
CGCGGCGAGCAGCTCTTCAGTGAAGAAGGAAGCAATCGGAGGGTCAGCAATGAACGTGGA 180
                                                  M  N  V  E
GCATGAGGTTAACCTCCTGGTGGAGGAAATTCATCGTCTGGGTTCCAAAAATGCCGATGG 240
  H  E  V  N  L  L  V  E  E  I  H  R  L  G  S  K  N  A  D  G
GAAACTGAGTGTGAAGTTTGGGGTCCTCTTCCAAGACGACAGATGTGCCAATCTCTTTGA 300
  K  L  S  V  K  F  G  V  L  F  Q  D  D  R  C  A  N  L  F  E
AGCGTTGGTGGGAACTCTGAAAGCCGCAAAACGAAGGAAGATTGTTACGTACGCAGGAGA 360
  A  L  V  G  T  L  K  A  A  K  R  R  K  I  V  T  Y  A  G  E
GCTGCTTTTGCAAGGTGTTCATGATGATGTTGACATTGTATTGCTGCAAGATTAATGTGG 420
  L  L  L  Q  G  V  H  D  D  V  D  I  V  L  L  Q  D
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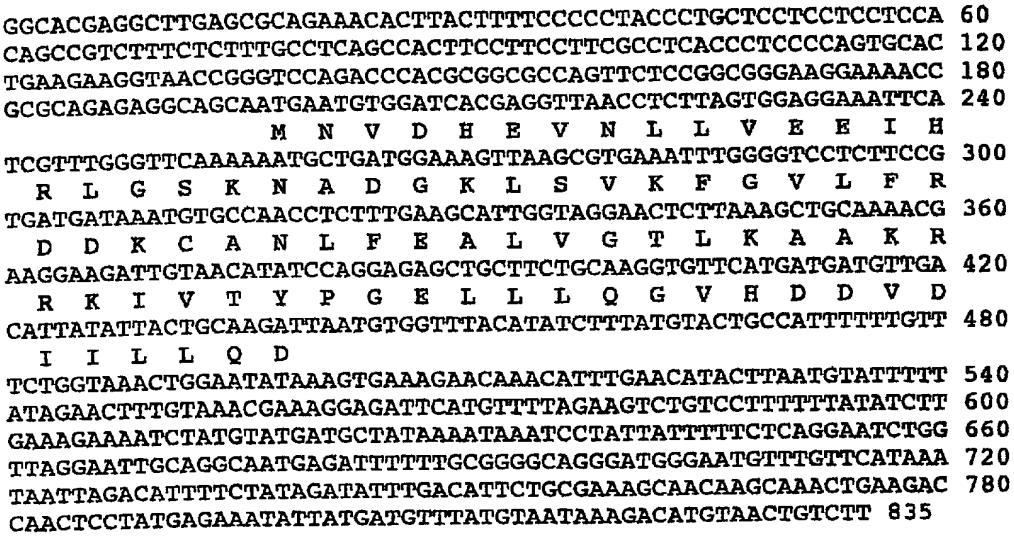
Figure 2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M N V D H E V N L L V E E I H
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R L G S K N A D G K L S V K F G V L F R
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D D K C A N L F E A L V G T L K A A K R
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R K I V T Y P G E L L L Q G V H D D V D
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I I L L Q D
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GAAAGAAAATCTATGTATGATGCTATAAAATAAATCCTATTATTTTTCTCAGGAATCTGG 660
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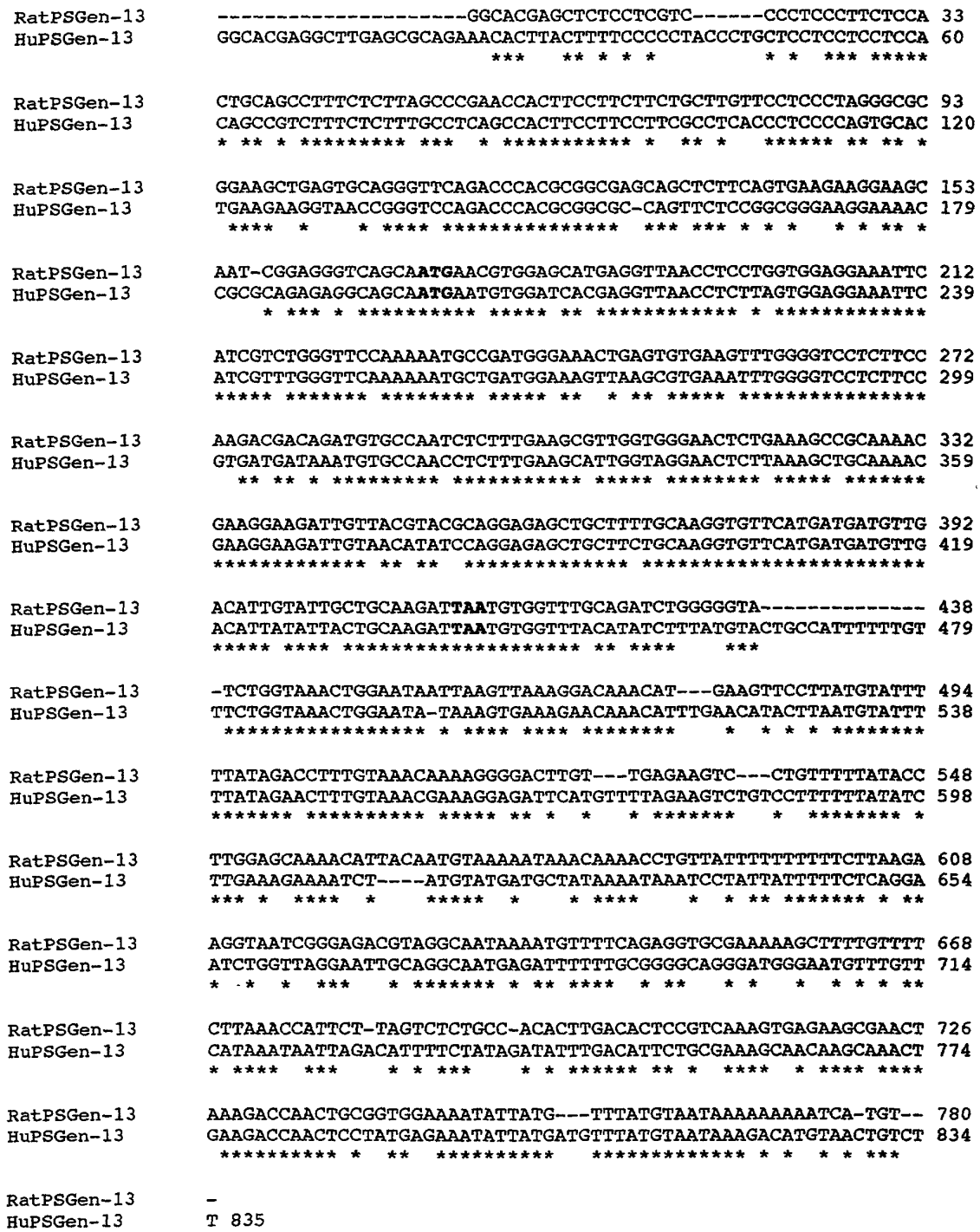
Figure 3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RatPSGen-13 CTGCAGCCTTTCTCTTAGCCCGAACCACTTCCTTCTTCTGCTTGTTCCTCCCTAGGGCGC 93
HuPSGen-13 CAGCCGTCTTTCTCTTTGCCTCAGCCACTTCCTTCCTTCGCCTCACCCTCCCCAGTGCAC 120
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HuPSGen-13 TGAAGAAGGTAACCGGGTCCAGACCCACGCGGCGC-CAGTTCTCCGGCGGGAAGGAAAAC 179
RatPSGen-13 AAT-CGGAGGGTCAGCAATGAACGTGGAGCATGAGGTTAACCTCCTGGTGGAGGAAATTC 212
HuPSGen-13 CGCGCAGAGAGGCAGCAATGAATGTGGATCACGAGGTTAACCTCTTAGTGGAGGAAATTC 239
RatPSGen-13 ATCGTCTGGGTTCCAAAAATGCCGATGGGAAACTGAGTGTGAAGTTTGGGGTCCTCTTCC 272
HuPSGen-13 ATCGTTTGGGTTCAAAAAATGCTGATGGAAAGTTAAGCGTGAAATTTGGGGTCCTCTTCC 299
RatPSGen-13 AAGACGACAGATGTGCCAATCTCTTTGAAGCGTTGGTGGGAACTCTGAAAGCCGCAAAAC 332
HuPSGen-13 GTGATGATAAATGTGCCAACCTCTTTGAAGCATTGGTAGGAACTCTTAAAGCTGCAAAAC 359
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HuPSGen-13 GAAGGAAGATTGTAACATATCCAGGAGAGCTGCTTCTGCAAGGTGTTCATGATGATGTTG 419
RatPSGen-13 ACATTGTATTGCTGCAAGATTAATGTGGTTTGCAGATCTGGGGGTA---------- 438
HuPSGen-13 ACATTATATTACTGCAAGATTAATGTGGTTTACATATCTTTATGTACTGCCATTTTTTGT 479
RatPSGen-13 -TCTGGTAAACTGGAATAATTAAGTTAAAGGACAAACAT---GAAGTTCCTTATGTATTT 494
HuPSGen-13 TTCTGGTAAACTGGAATA-TAAAGTGAAAGAACAAACATTTGAACATACTTAATGTATTT 538
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RatPSGen-13 TTGGAGCAAAACATTACAATGTAAAAATAAACAAAACCTGTTATTTTTTTTTTCTTAAGA 608
HuPSGen-13 TTGAAAGAAAATCT----ATGTATGATGCTATAAAATAAATCCTATTATTTTTCTCAGGA 654
RatPSGen-13 AGGTAATCGGGAGACGTAGGCAATAAAATGTTTTCAGAGGTGCGAAAAAGCTTTTGTTTT 668
HuPSGen-13 ATCTGGTTAGGAATTGCAGGCAATGAGATTTTTTGCGGGGCAGGGATGGGAATGTTTGTT 714
RatPSGen-13 CTTAAACCATTCT-TAGTCTCTGCC-ACACTTGACACTCCGTCAAAGTGAGAAGCGAACT 726
HuPSGen-13 CATAAATAATTAGACATTTTCTATAGATATTTGACATTCTGCGAAAGCAACAAGCAAACT 774
RatPSGen-13 AAAGACCAACTGCGGTGGAAAATATTATG---TTTATGTAATAAAAAAAAATCA-TGT-- 780
HuPSGen-13 GAAGACCAACTCCTATGAGAAATATTATGATGTTTATGTAATAAAGACATGTAACTGTCT 834
RatPSGen-13 -
HuPSGen-13 T 835

Figure 4

```
RatPSGen-13     MNVEHEVNLLVEEIHRLGSKNADGKLSVKFGVLFQDDRCANLFEALVGTLKAAKRRKIVT 60
HuPSGen-13      MNVDHEVNLLVEEIHRLGSKNADGKLSVKFGVLFRDDKCANLFEALVGTLKAAKRRKIVT 60
                ***:*******************************:**:**********************

RatPSGen-13     YAGELLLQGVHDDVDIVLLQD 81
HuPSGen-13      YPGELLLQGVHDDVDIILLQD 81
                *.***************:****
```

Figure 5

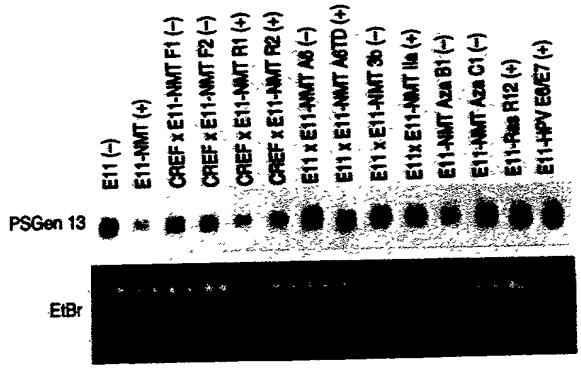

E11 (–)
E11-NMT (+)
CREF x E11-NMT F1 (–)
CREF x E11-NMT F2 (–)
CREF x E11-NMT R1 (+)
CREF x E11-NMT R2 (+)
E11 x E11-NMT A9 (–)
E11 x E11-NMT A9TD (+)
E11 x E11-NMT 3b (–)
E11x E11-NMT IIa (+)
E11-NMT Aza B1 (–)
E11-NMT Aza C1 (–)
E11-Ras R12 (+)
E11-HPV E6/E7 (+)
PSGen 13
EtBr

Figure 6

PSGen 13 Suppresses the Transformed Phenotype in E11-NMT Cells

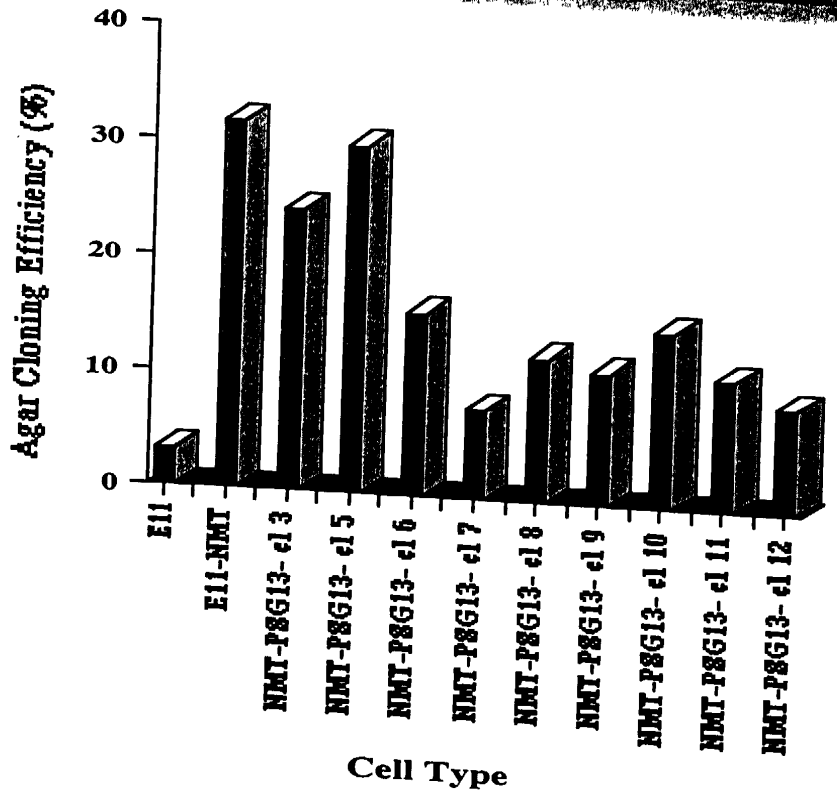


Figure 7

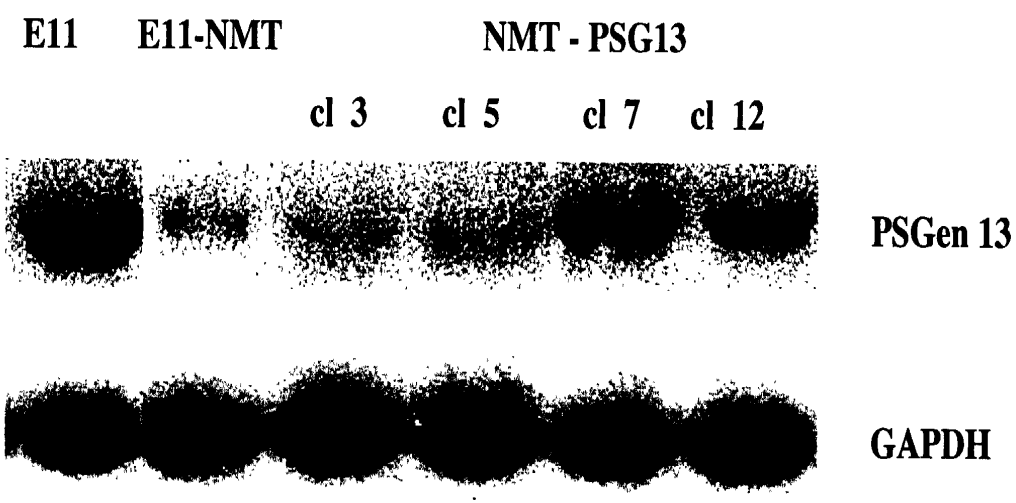


Figure 8

Rat PSGen 13 Inhibits Anchorage Independent Growth in DU-145 Cells

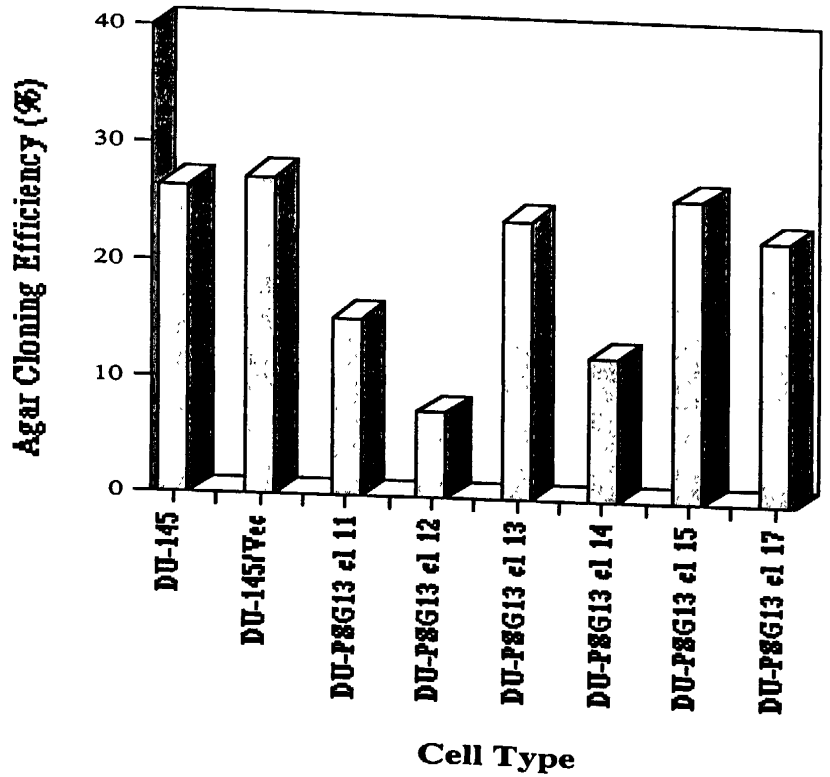


Figure 9

PSGen 13 Suppresses PEG-3 Promoter Activity in E11-NMT Cells

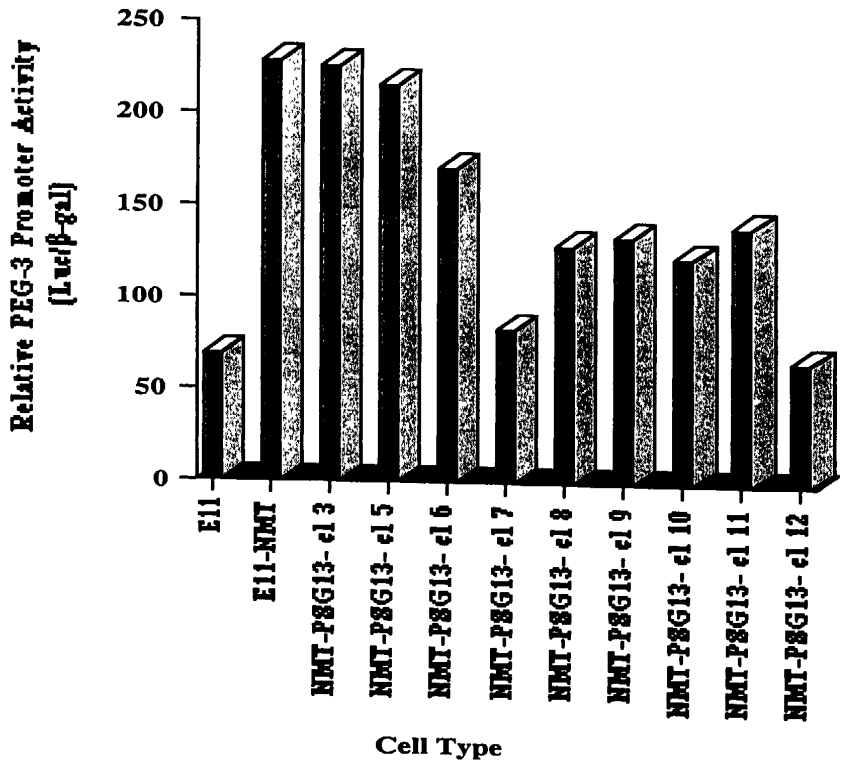


Figure 10

PSGen 13 Suppresses VEGF Promoter Activity in E11-NMT Cells

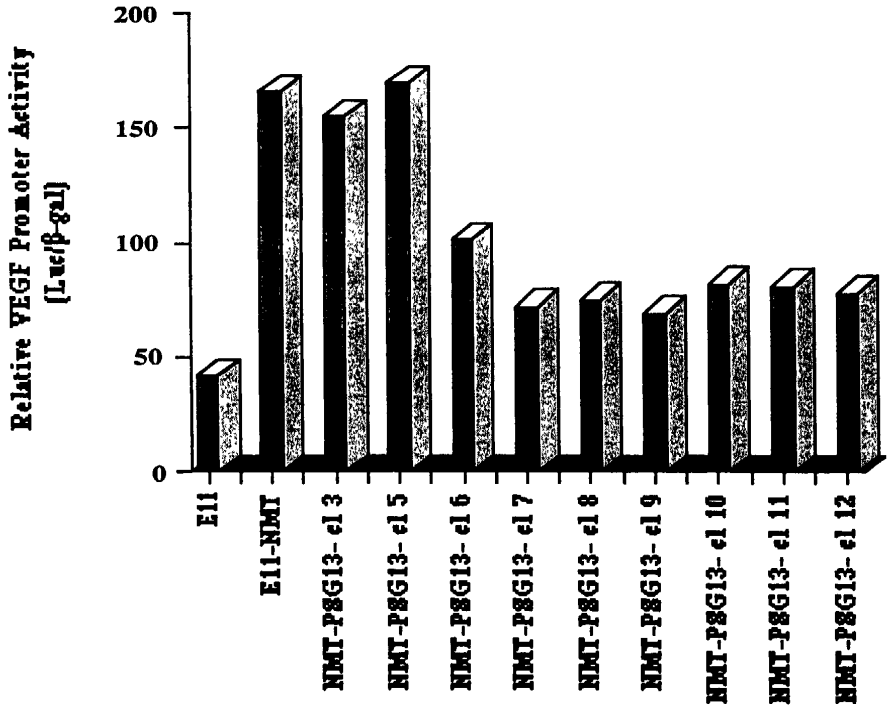


Figure 7

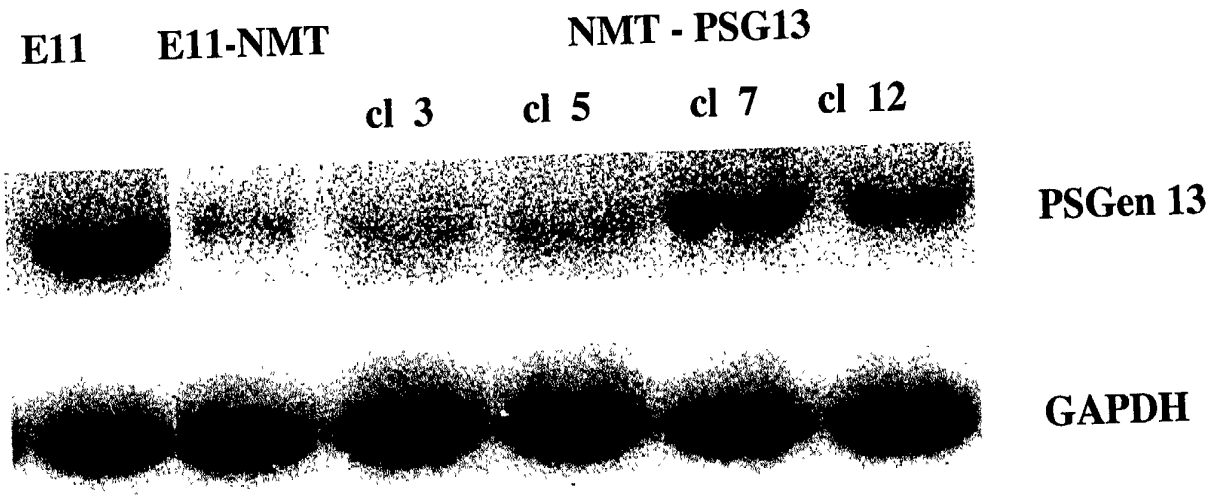


Figure 8

Rat PSGen 13 Inhibits Anchorage Independent Growth in DU-145 Cells

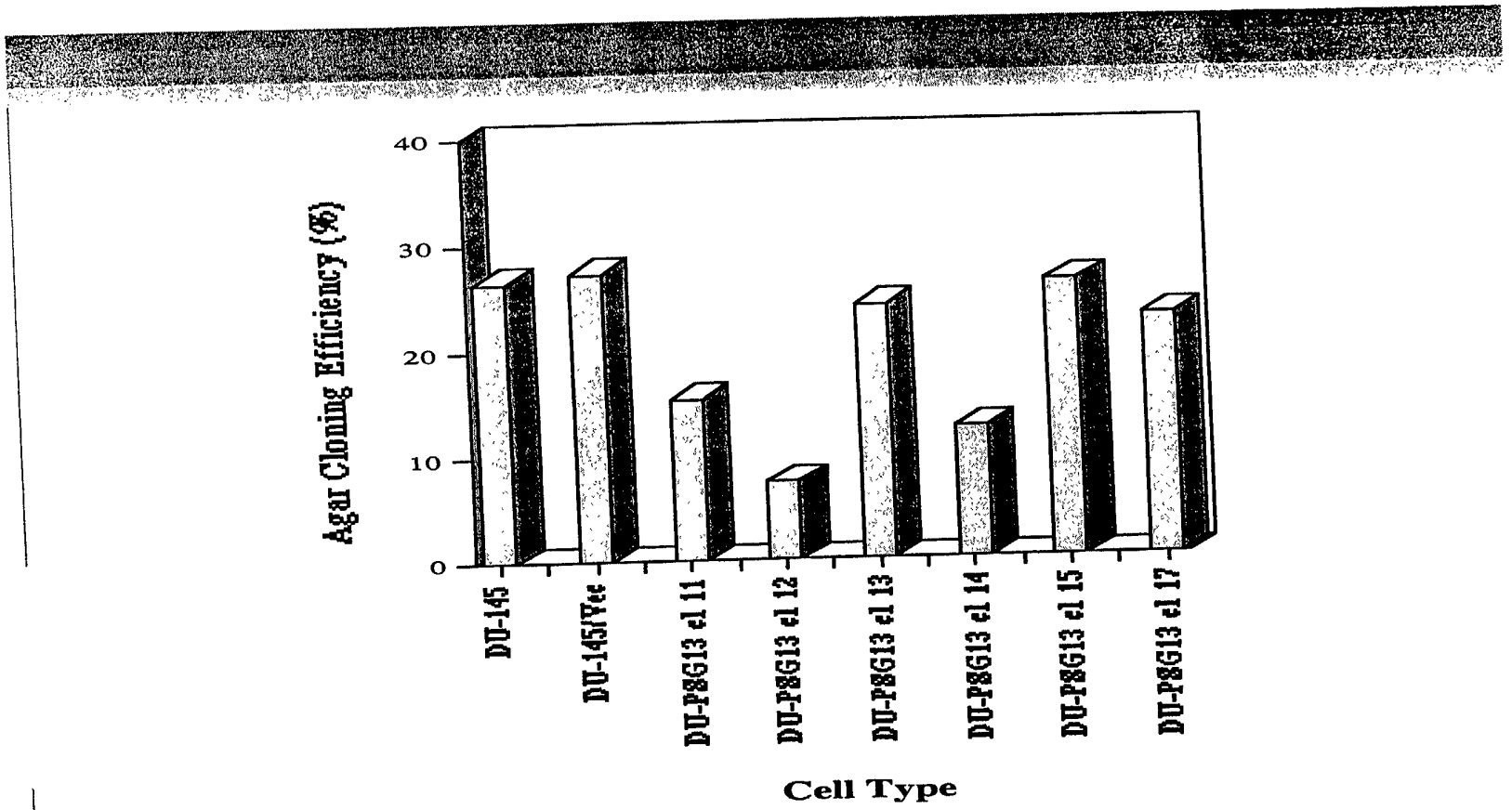


Figure 9

PSGen 13 Suppresses PEG-3 Promoter Activity in E11-NMT Cells

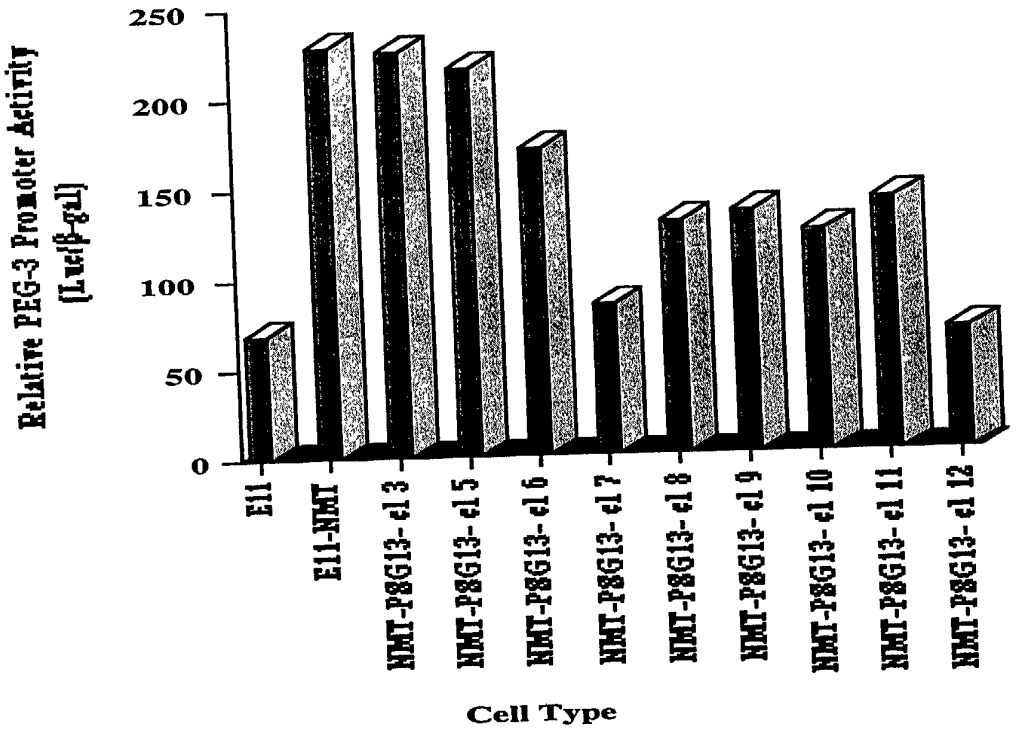
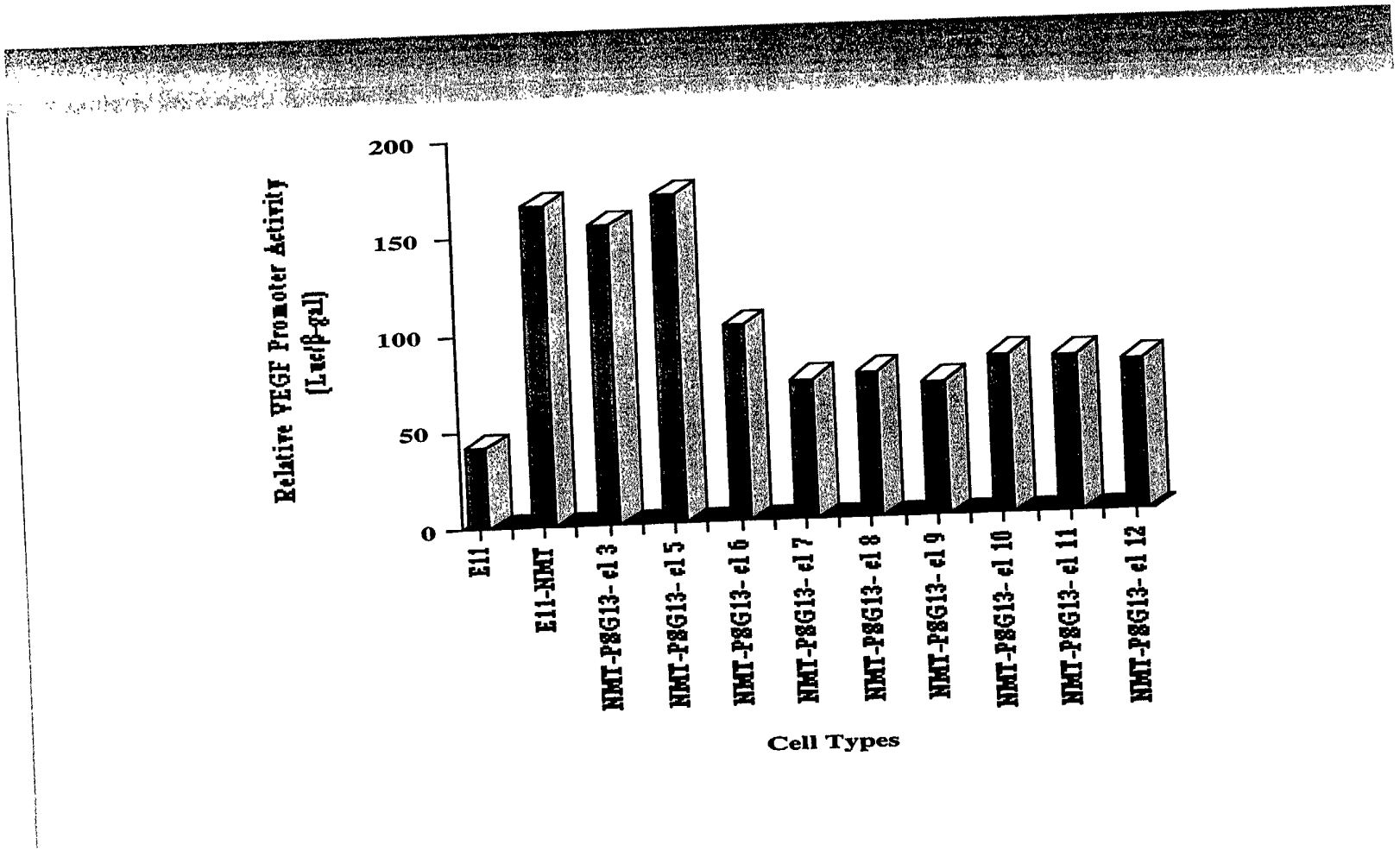


Figure 10

PSGen 13 Suppresses VEGF Promoter Activity in E11-NMT Cells



dkt. 62943/JPW/JML

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROGRESSION SUPPRESSED GENE 13 (PSGen 13) AND USEES THEREOF

the specification of which:
(check one)

X *is attached hereto.*

___ *was filed on* _____ *as*

Application Serial No. _____

and was amended _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
N/A			___	___
			___	___
			___	___
			___	___
			___	___
			___	___

Declaration and Power of Attorney *Page 2*

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

Provisional Application No.	*Filing Date*	*Status*
N/A		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:

Application Serial No.	*Filing Date*	*Status*
N/A		

And I hereby appoint

John P. White (Reg. No. 28,678); Christopher C. Dunham (Reg. No. 22,031); Norman H. Zivin (Reg. No. 25,385); Jay H. Maioli (Reg. No. 27,213); William E. Pelton (Reg. No. 25,702); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Wendy E. Miller (Reg. No. 35,615); Richard S. Milner (Reg. No. 33,970); Robert T. Maldonado (Reg. 38,232); Paul Teng (40,837); Richard F. Jaworski (Reg. No. 33,515); Elizabeth M. Wieckowski (Reg. No. 42,226); Pedro C. Fernandez (Reg. No. 41,741); Gary J. Gershik (Reg. No. 39,992); Jane M. Love (Reg. No. 42,812); Spencer H. Schneider (Reg. No. 45,923) and Raymond A. Diperna (Reg. No. 44,063).

and each of them, all c/o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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